UNIVERSIDADE DE LISBOA
FACULDADE DE MEDICINA DENTÁRIA

STUDY CONTRIBUTION FOR PERI-IMPLANT INFLAMMATORY RESPONSE AROUND DIFFERENT BIOMATERIALS IMUNOHISTOCHEMICAL STUDY SHEEP MODEL AND HUMAN RANDOMIZED CLINICAL CONTROL TRIAL

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ANDRÉ TSOU CHEN

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This thesis was undertaken as a requirement for the Degree of Doctor in Medicine with a specialization in Oral Surgery

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This thesis is dedicated to the Chen clan.... my parents, Maria (Mã Aka. Vóvó) and Rui (Pá Aka Vôvô), my sister Sara (Titi), brother-in law Miguel (Miné),

my beautiful wife Elena (mummy) and my three young "aprendices" Sofia (Sofy), Henrique (Kiké) and Rodrigo (Rodry)

With love

André (Papi)

“*Every Journey Begins with a small step*” – Sun Tzu, The Art of War

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No matter what happens, when you read these lines, remember how happy we were in June 2018, and smile …

Thesis Abstract

Objectives

To study the inflammatory/autoimmune interleukin (IL) reaction of peri-implant tissues to three different computer-aided designed and manufactured (cad-cam) biomaterials: (zirconia (Z), acrylic (A) and titanium (T) at different time frames: at baseline – T0 (both animal and human study), at 1 month - T1 (animal study) and T3 (at 8 weeks in the human study and at 3 months in the animal study).

To answer the question, “which material evokes a stronger inflammation response in peri-implant tissues” (measured in concentrations of interleukin 1β (IL-1β) and interleukin 6 (IL6)), two studies were undertaken.

The first, an animal trial study, whose primary goal was to determine IL concentration variations between time frames, refine cytokines extraction methodologies and calculate sample size for the second, A Human Randomized Clinical Trial. (RCT)

Part 1: Animal Study

Materials and methods:

Six adult male sheep each randomly received six mandibular titanium, platform-switch, implants (Biomet-Zimmer® 4.1/ 3.5x8,5 mm height), 1 mm below the crestal bone, three on each side, placed in the diastema (anatomical part of sheep mandible), between incisors and molars.

Peri-implant crevicular fluid (PICF) was analyzed for cytokines expression.

At the time of surgery each implant was randomly assigned a two-piece healing abutment made from milled CAD-CAM Titanium (T), milled CAD-CAM Zirconia (Z) or milled CAD-CAM Acrylic (A).

PICF samples were taken, on the day of surgery (1 hour after surgery was finished - baseline- T0), at 1 month (T1) and at three months (T3) with adsorbent paper (Periopaper®).

Samples were placed in Eppendorf tubes, transported in dry ice and stored at -80 ºC.

Interleukins IL-1β and IL6 were measured from validated ELISA kits (Raybiotech®).

Calibration Curve for IL6 and IL-1β and spectrophotometry at 450 nm readings were taken. In some samples concentration readings were randomly duplicated and triplicated for accuracy.

For the control group, the amount of IL-1β and IL6 was measured from periodontal crevicular fluid (PCF) sulcus of adjacent teeth (at T0 and T3), and the amount of IL from blood samples (BF) immediately taken after the first incision.

At each time point (baseline, T1 and T3) for IL6 and IL-1β, non-parametric tests were undertaken. *p* values<0,05 were considered statistically significant.

Results:

By Interleukin (IL6, IL-1β):

For IL6 at T0 (*p*=0,597), T1 (*p*=0,497) and T3 (*p*=0,481), and for IL-1β at T0 (*p*=0,857), T1 (*p*=0, 357) and T3 (*p*=0,237) there were no statistically significant differences between T, Z and A, regarding changes in IL expression (*p* values>0,05).

By Material (T, A, Z):

For each material (T (*p*=1,000); Z (*p*=0,857); A (*p*=0,095)), there were no statistically significant differences in IL6 (Mann-Whitney) between T0 and T3. For IL-1β there was also no significant difference, T (*p*=1,000); Z (*p*=0,905); A (*p*=0,286) respectively (*p* values>0,05).

By Time Frame (T0, T1, T3):

There were no statistical differences between (PCF) and (PICF), at baseline (*p*=0,688) and at day 3 (*p*=1,000) for IL6 (*p* values>0,05).

There were no statistical differences between (PCF) and (PICF), at baseline (T *p*=0,688, Z *p*=0,688, A *p*=1,000) and at day 3 (T *p*=1,000, Z *p*=1,000, A

p=0,125) for IL-1β (*p* values>0,05).

For T, Z and A, at T0 (corresponding to 1hour after surgery was over) all the parameters were equal, except that there was significantly less expression of IL6 (*p*=0,031<0,05) in PICF, compared to IL6 present in blood (BF), at the time of incision, for all abutments.

For IL-1β**,** all the parameters were equal with the exception of significantly less expression for Z (*p*=0,031<0,05) in PICF at T0 (1 hour after), than IL-1β present in blood fluid (BF), but there was no difference for A (*p*=0,375>0,05) or T (*p*=0,219>0,05).

Conclusions Animal Study:

With regard to expression of IL6 and IL-1β from T0 to T3 in the sheep animal model, all biomaterials (T, Z and A) exhibited similar behavior over time.

All samples expressed the same amount of IL with no differences for PIC of the adjacent teeth (at the same time frames).

For IL6 at T0, T, Z and A expressed lesser amounts of IL than IL6 present in BF.

Z abutment had a significant inferior IL-1β and IL6 expression than that present in the BF at T0.

The lower reaction triggered by Z abutments (measured in osteoclastic inducer IL-1β) but not by A or T, may be the key to understanding if different materials have different inflammatory patterns in the first phases of healing.

Part 2: Human RCT

Objective

To study the inflammatory interleukin reaction of peri-implant tissues to different Biomaterials (zirconia, acrylic and titanium) at different time frames (baseline T0, and at 8 weeks T2).

Materials and Methods

Clinical Trials Registry was done at http://clinicaltrials.gov under the registered name Implantology Institute, Portugal, and was assigned the number **NCT01961635** for free consultation.

The Clinical trial was reported according to the CONSORT statement ® for a parallel randomized non-inferiority clinical controlled trial.

Three Arms were used (60 subjects - 20 in each arm) with a common surgical phase – place platform-switch dental Implants (Biomet-Zimmer® 4.1/3.5) subcrestally, and then three (with different materials) randomly placed, two-piece healing abutments (CAD-CAM zirconium oxide (Z), CAD-CAM commercially pure titanium IV (T) or polimetacrilate CAD-CAM processed Acrylic (A)).

The primary objective was to evaluate changes in inflammatory levels (measured in IL6 and IL-1β) from T0 (baseline) to T2 (8 weeks).

Secondary outcomes such as marginal bone loss (MBL), gingival height (GH) levels, osseointegration, gender, age, time of surgery, anatomical position and implant stability were also taken into account in the evaluation.

All *p* values<0,05 were considered statistically significant.

Results

IL6 and IL-1β inflammatory results by time frame (T0, T2)

At T0, IL-1β (5,24 ± 3,91 pg/ml), IL6 (6,20 ± 5,43 pg/ml) and total IL-1β + IL6 (11,44 ± 7,62 pg/ml), did not differ significantly with the material. (Z, T or A).

Only IL-1β (55,41 ± 49,85 pg/ml) differed significantly, with the material at T2. Analyzing pairwise comparisons at T2, IL-1β differed significantly between T and Z (29,94 ± 54,0764 pg/ml for Z vs 75 ± 55,24 pg/ml for T), with IL-1β being, on average, significantly higher in T. In the other 2 pairs (Z -29,94 ± 54,07 pg/ml vs A -31,44 ± 33,40 pg/ml and T-A) there were no statistically significant differences.

Results by Material (Z, A, T)

For T (4,65 ± 4,57 pg/ml at T0 and 4,06 ± 7,99 pg/ml at T2), and A (7,63 ± 6,58 at T0 and 8,56 ± 14,82 pg/ml at T2) the results were very similar, showing that for IL6 there were no statistically significant differences between T0 and T2.

For IL-1β, concentrations were significantly higher at T2, for both material T (6,35 ± 5,37 pg/ml at T0 and 64,75 ± 55,24 pg/ml at T2) and A (5,31 ± 3,16 pg/ml at T0 and 31,44 ± 33,40 pg/ml at T2).

Using Z, IL-1β (4,11 ± 2,7 pg/ml at T0 and 29,94 ± 54,07 pg/ml at T2) was significantly higher at T2, but the IL6 values were not (6,17± 4,64 pg/ml at T0 and 4,76 ± 13,83 pg/ml at T2).

Results PICF (peri-implant crevicular fluid) vs PCF (periodontal crevicular) vs BF (blood fluid)

PCF extracted from the adjacent control teeth was on average -2,4 pg/ml for IL6 (which as a biological measure was considered 0 or absence of IL) and 15,15 pg/ml for IL-1β.

In relation to PICF for IL6 concentrations in all implants (54 readings), independent of the material (A, Z or T) it was concluded that at T0, the total IL6 present in PICF was, on average, significantly higher than the value of the PCF.

At T2, the IL6 of PICF was again, on average, also significantly higher than the value of PCF.

In relation to PICF for IL-1β concentrations in all implants (54 readings) independent of the material (A, Z or T), it was concluded that at T0, IL-1β was, on average, significantly lower than the value of IL-1β of PCF of the adjacent teeth, but at T2, IL-1β present at PICF, was on average, significantly higher than the value of IL-1β present in PCF. In terms of the other control group, the blood fluid at the time of incision (BF), results showed that, when analyzed by time frame, at T0, IL6 was, on average, significantly higher than the BF, but IL-1β was not.

At T2, IL6 and IL-1β present in the PICF were, on average, significantly higher than the BF. Analyzing BF and compared to PICF of different material, the

results showed that, Z at T0, in relation to IL6 expression was, on average, significantly higher than the BF, but not IL-1β, which had a lower value on PICF in the same time frame.

At T2 for the Z abutment, all IL levels of the PICF were higher than IL levels of BF.

For T and A at T0, PICF IL6 expression was higher than BF and IL-1β showed no statistical differences. At T2, for A and T, the IL6 and IL-1β expression was higher in PICF than in the BF values.

Results: MBL (Marginal Bone Loss) vs Inflammation

Athough there was a tendency for the Z healing abutment to have less MBL, no statistical differences between MBL on the three healing abutments were found.

In our results, there was a tendency for there to be less MBL when there was less expression of PICF IL-1β on the Z healing abutment. (8,79 ± 13,13 mm for T, 8,67 ± 9,04 mm for A and 5,65± 7,91 mm for Z).

No correlation between MBL (measured at T2) and the concentration of IL measured at T0 or at T2 was found, leading to the conclusion that none of the three biomaterials was more or less correlated with marginal bone resorption.

Secondary Results Outcomes: to relate inflammatory levels to MBL and Height of gingiva

Initial gingival height did not significantly influence MBL. In terms of inflammation, height significantly influenced the values of IL6 at T0 (2,87 ± 4,03 pg/ml for 2 mm and 7,41 ± 5,40 pg/ml for 3 mm) and IL-1β (4,25 ± 4,68 pg/ml for 2 mm vs 5,50 ± 3,53 pg/ml for 3mm), where inflammatory markers were on average significantly higher in a 3 mm tissue height than in those of 2 mm.

In all indicators, at T2, height did not significantly influence IL-1β, IL6 and total indicators, indicating that MBL and inflammatory levels did not correlate with the height of pre-existing tissue in our study sample.

Age (≥65 and <65)

In relation to age, results showed that at T2 (8 weeks), age did not significantly influence IL-1β, IL6 and IL6+IL-1β.

At T0, IL6 differed significantly with age, and, on average, IL6 was significantly higher at ≥65 years (4,45 ± 4,54 pg/ml vs 8,57 ± 5,71 pg/ml). The same conclusions apply for IL-1β (4,16 ± 2,67 pg/ml vs 6,69 ± 4,83 pg/ml), indicating that at T0 patients older or equal to 65 tended to experience more inflammation (IL6, IL-1β and IL-1β +IL6) at early stages of implant placement than patients under 65 years old.

Gender (male vs female)

Inflammatory indicators at T2, IL-1β, IL6 and IL6+IL-1β do not differ significantly with gender. At T0, IL6, was on average, significantly higher in males (4,36 ± 4,23 pg/ml vs 45 ± 5,85 pg/ml.). The other indicators did not differ significantly with gender.

MBL is significantly influenced by gender: on average, women experienced more bone loss than men (mean 0,8 mm Vs 1,3 mm).

Anatomical position (maxilla vs mandible)

Correlating the 3 variables, MBL inflammation and biomaterials, results showed that MBL differed significantly with the position, and in the maxilla, bone loss was on average significantly higher (mean 0,92 vs 1,08 mm).

None of the 3 inflammatory indicators (IL6, IL-1β and total IL6 + IL-1β) at T2 differed significantly with position. The same conclusion was drawn for T0 (baseline).

Duration of surgery

Duration does not significantly influence marginal bone loss and there were no instances where the duration influenced the indicated inflammatory variables.

Stability Values

One of the first conclusions, was that at T0 (baseline), implant stability was not significantly related to MBL nor was it related to inflammation, namely to IL6, IL-1β and IL6+IL-1β.

When comparing anatomical position, we found that at T0, stability differed with position being, on average, significantly higher in the mandible than in the maxilla.

Conclusion

The autoimmune / inflammatory response exists moderately in dental implants.

Inflammatory indexes that are present in the PICF sulcus may be responsible for marginal bone loss, among other problems that can affect a dental implant.

IL-1β was expressed in greater quantity in titanium abutments at T2 (end of osseointegration process). However, in all implants, without exception, the concentration of IL at T2 was statistically higher than at T0.

This expression was not found in the control groups blood values (BF) nor in PCF of healthy teeth.

Placement of a dental implant into the oral cavity triggers a local inflammatory reaction that remains in a chronic form over time, very similar to a low density foreign body reaction.

The attribution of marginal bone loss solely to a bacterial phenomenon is to be seen, in the light of this research thesis, as a highly reductive explanation. The host response to a foreign body may play a pivotal role, as or more important than the microbiological theory of biological width formation.

Keywords: Inflammation, Auto-immune response, IL6, IL-1β, dental implants, CAD-CAM Zirconia, CAD-CAM Acrylic, CAD-CAM Titanium, Marginal Bone Loss.

Resumo da tese

Objetivos:

Estudar a reação inflamatória (medida em IL-1β e IL6) de tecidos peri-implantares, a diferentes biomateriais (cad-cam dióxido de zircónia, cad-cam acrílico e cad-cam titânio) em diferentes períodos de tempo (dia da cirurgia T0 a T1 – 1mês, a T3- 3 meses). Num estudo animal em modelo ovino.

Para responder á questão, qual o biomaterial, que em contacto com o tecido conjuntivo peri-implantar provoca menos inflamação (medida na concentração de IL-1β e IL6) elaborámos dois estudos.

Um estudo experimental animal, para determinar as variações das concentrações de IL entre estadios (T0, T1, T3), com objetivo de refinar as metodologias de extração de citoquinas e calcular o tamanho da amostra para o segundo estudo desta tese: o ensaio clínico aleatorizado humano (RCT)

Parte 1: Estudo animal em modelo Ovino

Materiais e métodos:

Seis ovelhas adultas, receberam cada uma, aleatoriamente, seis implantes de titânio, com plataforma discrepante (Biomet-Zimmer ® 4.1 / 3.5x8,5 mm), 1 mm abaixo do osso crestal, três de cada lado, colocados na zona denominada de "diastema" (parte anatómica da mandibula) que se situa entre incisivos e pré-molares da ovelha. O fluido crevicular peri-implantar (PICF) foi analisado para caracterizar a expressão de citoquinas.

No momento da cirurgia (T0), para cada implante, foi distribuído aleatoriamente, um pilar de duas peças de titânio, zirconia ou acrílico. Foram colhidas amostras de PICF, no dia da cirurgia (1 hora após a conclusão da cirurgia – T0), 1 mês (T1) e três meses (T3) com papel adsorvente (Periopaper®). As amostras foram colocadas em tubos de eppendorf, transportadas em gelo seco e armazenadas em -80º C.

As concentrações de interleucinas 1β (IL-1β) e 6 (IL6) presentes em cada amostra, foram medidas a partir de kits *ELISA* validados (Raybiotech®)

As curvas de calibração para IL6 e IL-1β e espectrofotometria a 450 nm foram feitas. Duplicámos aleatoriamente e triplicámos cada amostra para obter precisão.

Para o grupo de controlo, medimos a quantidade de IL-1β e IL6 presente no fluido crevicular periodontal (PCF) do sulco de dentes adjacentes (em T0 e T3) e a quantidade de IL em amostras de sangue (BF), imediatamente tomadas após a primeira incisão, no dia da cirurgia.

Em cada ponto de medição para IL6 e IL-1β, um teste não paramétrico foi utilizado. p <0,05 foram considerados estatisticamente significativos.

Resultados:

Considerando a variação de interleucinas por tempo, para a IL6 em T0 (p = 0,597), T1 (p = 0,497) e T3 (p = 0,481), e para a IL-1β em T0 (p = 0,857), T1 (p = 0,357) e T3 (p = 0,237) não houve diferenças estatisticamente significativas entre T, Z e A, em relação á variações na expressão de interleucinas (p> 0,05).

Para cada material, não houve diferenças estatisticamente significativas (Mann-Whitney) entre T0 e T3 para IL6, T (p = 1.000); Z (p = 0,857); A (p = 0,095) e para IL-1β, T (p = 1,000); Z (p = 0,905); A (p = 0,286) respetivamente. (p> 0,05)

Não houve diferenças estatisticamente significativas, entre fluido crevicular periodontal (PCF) e perimplantar (PICF), em T0 (p = 0,688) e em T3 (p = 1,000) para IL6 (p> 0,05)

Não houve diferenças estatisticamente significativas entre PCF e PICF, em T0 (T p = 0,688, Z p = 0,688, A p = 1.000) e em T3 (T p = 1.000, Z p = 1,000, A p = 0,125) para IL-1β (p> 0,05)

Para T, Z e A, em T0 houve menor expressão de IL6 (p = 0,031 <0,05) em PICF, do que a IL presente no sangue (BF), no momento da primeira incisão.

Para IL-1β houve menor expressão de concentração no pilar de Z (p = 0,031 <0,05) no PICF em T0 (1 hora depois da cirurgia ter acabado), do que a concentração de IL presente no sangue (BF) no momento da incisão. O mesmo não se passou para os pilares de A (p = 0,375> 0,05) e de T (p = 0,219> 0,05) que se mantiveram com concentrações semelhantes.

Conclusões Estudo Animal:

Em relação à expressão de IL6 e IL-1β de T0 para T3, os pilares de T, Z e A têm um comportamento semelhante, expressando a mesma quantidade de IL ao longo do tempo.

Expressam também a mesma quantidade de IL do que o grupo de controlo (PIC de dentes adjacentes), num período de tempo similar.

Para IL6 em T0, os pilares de T, Z e A expressam menor quantidade de IL, do que a mesma IL presente no sangue colhido aquando da primeira incisão.

O pilar de Z tem uma expressão inferior de IL-1β e IL6 (estatisticamente significativa) do que a IL presente no sangue (BF) em T0.

A reação inflamatória menor provocada pelos pilares de Z (medida em IL-1β), mas não de A ou T, pode ser a chave para entender, se diferentes materiais possuem diferentes padrões inflamatórios nos primeiros dias de cicatrização peri-implantar.

Parte 2: Ensaio Clínico Aleatorizado (RCT) humano

Objetivo

Estudar a reação inflamatória (medida em IL-1β e IL6) de tecidos peri-implantares, a diferentes biomateriais (cad-cam zircónia, cad-cam acrílico e cad-cam titânio) em diferentes períodos de tempo (dia da cirurgia T0 a T2- 8 semanas após a cirurgia). Em humanos, num ensaio clínico aleatorizado.

Materiais e métodos

O ensaio clínico foi registado em Clinical Trials Registries, http://clinicaltrials.gov sob o nome Implantology Institute, Portugal, recebendo o número **NCT01961635** para consulta livre.

Ensaio clínico elaborado de acordo com a declaração CONSORT ® para ensaios clínicos paralelos aleatorizados, de não inferioridade.

Três grupos de estudo (60 pacientes - 20 em cada grupo) com uma fase cirúrgica comum - colocar implantes dentários de plataforma discrepante

(Biomet-Zimmer® 4.1/3.5) subcrestal, e três pilares de cicatrização de duas peças diferentes, óxido de zircónio (Z), titânio grau IV (T), polimetacrilato CAD-CAM Acrílico (A).

Avaliar as alterações nos níveis inflamatórios de T0 (baseline) para T2 (8 semanas após cirurgia). Avaliar também os resultados das variáveis secundárias: perda óssea marginal (POM), níveis de altura gengival (GH), osteointegração, género, idade, tempo de cirurgia, posição anatómica e estabilidade do implante.

Resultados com p <0,05 foram considerados estatisticamente significativos.

Resultados

Resultados da resposta inflamatória por tempo (T0, T2)

Em T0, a IL-1β (5,24 ± 3,91 pg / ml), a IL6 (6,20 ± 5,43 pg / ml) e o valor total (IL6 + IL-1β) 11,44 ± 7,62 pg / ml, não diferiram significativamente com o material. (Z, T ou A).

Apenas a IL-1β (55,41 ± 49,85 pg / ml) diferiu, em T2, significativamente, com o material. Analisando as comparações em pares, em T2, a IL-1β diferiu significativamente entre T e Z (29,94 ± 54,0764 pg / ml versus 75 ± 55,24 pg / ml), sendo a IL-1β, em média, significativamente maior em T. Os outros 2 pares (Z-29,94 ± 54,07 pg / ml vs A- 31,44 ± 33,40 pg / ml e T-A) não houve diferença estatisticamente significativa.

Resultados por Material (Z, A, T)

Para o pilar de T (4,65 ± 4,57 pg / ml T0, 4,06 ± 7,99 pg / ml T2) e A (7,63 ± 6,58 T0, 8,56 ± 14,82 pg / ml T2) os resultados foram muito semelhantes, no que concerne à IL6 não existiram diferenças entre T0 e T2.

Para a IL-1β, a concentração foi significativamente maior em T2 para o material T (6,35 ± 5,37 pg / ml a T0-64,75 ± 55,24 pg / ml em T2) e A (5,31 ± 3,16 pg / ml a T0 e 31,44 ± 33,40 pg / ml em T2).

Relativamente ao uso de Z, a concentração de IL-1β (4,11 ± 2,7 pg / ml T0,

29,94 ± 54,07 pg / ml) foi significativamente maior em T2, mas os valores de IL6 não (6,17 ± 4,64 pg / ml T0 e 4,76 ± 13,83 pg / ml T2)

Resultados PICF (fluido crevicular peri-implantar) vs PCF (fluido crevicular periodontal) vs. BF (fluido sanguíneo)

O PCF foi em média -2,4 pg / ml para IL6 (o que considerámos como valor biológico 0, ou seja, ausência de interleucina) e 15,15 pg / ml para IL-1β.

Considerando o PICF das concentrações de IL6, em todos os implantes (54 leituras) independentes do material (A, Z ou T), concluímos que em T0, a IL6 total foi, em média, significativamente maior que o valor do PCF.

Em T2, a IL6 do PICF foi, em média, novamente, significativamente maior do que o valor de PCF.

Considerando o PICF das concentrações de IL-1β, em todos os implantes (54 leituras) independentes do material (A, Z ou T), concluímos que em T0, a IL-1β foi, em média, significativamente menor do que o valor do PCF do dente, mas em T2, o PICF da IL-1β, foi, em média, significativamente maior que o valor de PCF.

Para as amostras de sangue (BF) e analisando pelos intervalos de tempo (T0 para T2) verificamos que, em T0, a IL6 foi, em média, maior do que os valores de BF.

Em T2, os valores de concentração de IL6 e da IL-1β foram, em média, significativamente maiores que o BF.

Analisando BF por material, os resultados mostram que, no pilar de Z a concentração de IL6 no PICF em T0, foi, em média, significativamente maior do que a IL6 presente no BF, em relação á IL-1β para a mesma comparação não existiram diferenças estatisticamente significativas. Em T2 todos os valores de inflamação PICF são mais elevados do que a concentração no BF.

Para T e A em T0, IL6 teve valores de concentração mais elevados do que BF, mas a IL-1β não teve diferença estatisticamente significativa. Em T2 a IL6 e a IL-1β também foram estatisticamente maiores do que os valores BF.

Resultados perda óssea marginal (POM) vs. Inflamação

Existiu tendência para o pilar de cicatrização de Z induzir menor perda óssea marginal, apesar dessa tendência, não encontrámos diferenças estatisticamente significativas entre eles.

Os resultados mostraram uma tendência para o pilar de Z induzir uma inferior POM, ao mesmo tempo que expressa menor quantidade de IL-1β. (8,79 ± 13,13 mm para T, 8,67 ± 9,04 para A e 5,65 ± 7,91 para Z)

Neste trabalho experimental, não encontrámos correlação entre POM (medida em T2) e a concentração de IL medida em T0 e T2. Nenhum dos três biomateriais correlacionou-se mais ou menos, à reabsorção óssea marginal.

Resultados das variáveis secundárias (impacto nos mediadores inflamatórios e de remodelação óssea marginal)

Espaço Biológico Residual (altura gengival)

A altura gengival não influenciou significativamente a POM. Quando se trata de inflamação, em T0, a altura influenciou significativamente os valores de IL6 (2,87 ± 4,03 para 2 mm e 7,41 ± 5,40 para 3 mm) e IL-1β (4,25 ± 4,68 para 2 mm vs. 5,50 ± 3,53 para 3mm), em média, esses indicadores foram significativamente maiores em 3 mm de altura de tecido gengival do que nos casos onde existia apenas 2 mm.

Em todos os indicadores, em T2, a altura não influenciou significativamente a IL-1β ou a IL6. Significando que POM e níveis inflamatórios não se correlacionaram com a altura do tecido pré-existente neste estudo.

Idade (≥65 e <65)

Como resultados, verificámos que em T2 (8 semanas), a idade não influenciou significativamente os valores de IL-1β, IL6. Em T0, a IL6 diferiu significativamente com a idade e, em média, a IL6 foi significativamente maior em ≥ 65 anos (4,45 ± 4,54 pg / ml versus 8,57 ± 5,71 pg / ml).

As mesmas conclusões para a IL-1β (4,16 ± 2,67 pg / ml vs 6,69 ± 4,83 pg / ml). O que significa que em T0 as pessoas com idade acima ou igual a 65 tendem a expressar maior inflamação (IL6, IL-1β e IL-1β+IL6) nos estágios iniciais da colocação do implante, que as pessoas com menos de 65 anos de idade

Género (masculino vs. feminino)

Em relação aos indicadores inflamatórios em T2, os indicadores da IL-1β, da IL6 e da IL6+ IL-1β não diferiram significativamente com o género. Em T0, a IL6 foi, em média, significativamente maior no género masculino (4,36 ± 4,23 pg / ml, 45 ± 5,85 pg / ml). Os outros indicadores não diferiram significativamente com o género.

A POM foi significativamente influenciada pelo gênero: em média, as mulheres apresentaram maior perda óssea do que os homens (média de 0,8 mm Vs. 1,3 mm).

Posição anatómica (maxila vs. mandíbula)

Apenas a POM diferiu significativamente com a posição, na maxila, a perda óssea foi, em média, significativamente maior (média de 0,92 vs. 1,08 mm). Nenhum dos 3 indicadores inflamatórios (IL6, IL-1β e IL6 + IL-1β total) em T2 diferiu significativamente com a posição. A mesma conclusão para T0 (baseline)

Duração da cirurgia

A duração não influenciou significativamente a perda óssea marginal e, em nenhum caso, a duração influenciou as variáveis inflamatórias indicadas.

Valores de Estabilidade ISQ

Uma das primeiras conclusões é que em T0 (baseline), a estabilidade do implante não esteve significativamente relacionada à POM nem esteve

relacionada com inflamação (IL6, IL-1β e IL6 + IL-1β).

Ao comparar a posição anatómica, descobrimos que, em T0, a estabilidade diferiu com a posição, sendo, em média, significativamente maior na mandíbula do que na maxila.

Conclusões

A resposta autoimune/inflamatória existe de uma forma moderada nos implantes dentários. Os índices inflamatórios que estão presentes no sulco PICF podem ser responsáveis por perda óssea marginal e outros problemas biológicos.

A IL-1β é expressa em maior quantidade nos pilares de titânio em T2, correspondendo á etapa final da osteointegração. No entanto em todos os implantes, sem exceção, a concentração de IL em T2 é estatisticamente superior ao encontrado em T0.

Essa expressão não foi encontrada nos valores sanguíneos de controlo nem no periodonto são.

A colocação de um implante dentário na cavidade oral despoleta uma reação inflamatória local que se mantêm de uma forma cronica muito semelhante a uma reação de corpo estranho de baixa densidade.

A atribuição de perda óssea marginal unicamente a um fenómeno bacteriano é á luz desta tese de investigação redutor. A resposta do hospedeiro a um corpo estranho pode desempenhar um papel fulcral tão ou mais importante do que que a teoria microbiológica de formação de espaço biológico livre.

Palavras-chave: Inflamação, resposta autoimune, IL6, IL-1β, implantes dentários, Zirconia CAD-CAM, Acrílico CAD-CAM, Titânio CAD-CAM, Perda óssea marginal

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LIST OF ABBREVIATIONS

% Percentage

- Number

SD - Standard deviation

vs. - Versus

T - Titanium

Z - Zirconia

CAD-CAM - Computer aid design Computer aid manufactured

A - Acrylic

IL-1β - interleukin 1β

IL6 - Interleukin 6

IL - Interleukin

mm - Millimeters

pg - Picogram

ml-Milliliter

ul-Microliter

I - Implant

min - Minutes

PCF - Periodontal Crevicular fluid

PICF - Peri-implant crevicular fluid

BF - Blood fluid levels

T0 – Baseline (both animal and RCT study)

T1 - 1 month (animal study only)

T2 - 2 month (RCT study only)

T3 - 3 Month (animal study only)

RCT – Randomized clinical control

MB - Marginal bone

MBL- Marginal bone loss

MBL1 - Marginal bone loss considering all implants

MBL2 - Marginal bone loss considering only the implants that had exposed surface

POM - Perda Óssea Marginal

Max - Maxila

Mand - Mandible

ISQ - Implant stability quotient

AS - Animal study

IST-Instituto Superior Técnico

FMDUL- Faculdade de Medicina Dentária da Universidade de Lisboa

ABC - Avidin-Biotin-Peroxidase Complex

BW- Biological width

R - Different time frames on sample readings

PBS - Buffer Solution

HRP-Conjugate

TMB – Termination solution

[ ] – Concentration

n - Number of cases

p - Statistical value of significance

PhD - Doctor of Philosophy

OD- Optical diffraction

H - Higher

L- Lower

S - Same

T - Time

M - Mesial

Ma - Material

D - Distal

Ave - Average

ML - Male

FM - Female

Psb - Primary stability

SS - Secondary stability

PS - Platform switching

CHAPTER 1. INTRODUCTION

1.1. HISTORICAL PERSPECTIVE

PI Brånemark's 25 years of osseointegration research in 1977(Brånemark et al. 1977) marked a revolution in dentistry, defining two separate eras of implant dentistry. (Adell et al. 1981a).

Before Brånemark, implant dentistry, had been characterized by clinicians using metal devices for fixed anchorage, with limited knowledge and investigation to support the use of dental implants.

The development of these types of devices for edentulous patients was taking place mainly in Europe and North America. Leonard Linkow from North America (Brinks, Kuyl, and Zeegers 1988) created several devices for bone anchorage to solve the retention problems of fully edentulous patients. His first work on implants was published in 1954, and was followed by 24 others, before the paper in which he presented his blades. (L. Linkow 1966)

Linkow was the first to produce a subperiosteal implant (L. I. Linkow 1967), a device that was placed between bone and periosteum to allow for the support of dentures and multiple elements.

Linkow's original subperiosteal implant was tailored to different shapes according to the anatomical site of insertion. (L. I. Linkow 1986)

The result was fair but this kind of approach had both poor and low survival rates which were highly prone to infection (L. I. Linkow and Ghalili 1998; L. I. Linkow 1967) and which didn't meet the full criteria for a fixed long term stability oral rehabilitation.

Motivated by the low survival rate of the subperiosteal implants, endosseous implants were making headway and later Linkow blades were created and published in 1966 (L. I. Linkow 1966), making it possible to treat partial or total edentulism.

The technique consisted of opening an access in bone to insert a titanium blade for single, partial or full mouth rehabilitation. This invention helped pave the way for the innovations in implant dentistry of the 1960s.

Pasqualini (Goutoudi, Diza, and Arvanitidou 2004) proposed a 'polymorphous'

blade implant, which could be modelled to meet the most common anatomical configurations and which, with its screw abutment, offered a solution to the problem of tongue-thrust during swallowing (causing the majority of post-surgical failures) for the first time.

Tramonte in Italy and Cherchève (Linkow co-wrote a two-volume work with the Frenchman Cherchève: Theories and Techniques of Oral Implantology) (Cherchève 1966) were also inventing screw-like implants that were inserted in bone to allow for fixed, stable restoration. (Passi et al. 2017)

Tramonte (S. Tramonte 1965) used screws, casted in chrome-cobalt, making them with a thinner profile and honing their threads to make them sharper.

These were machined dental implants with a higher survival rate than the subperiosteal or the blade implants of Linkow. (S. M. Tramonte 1989)

This period of research was characterized by strong personal experience, unpredictability of results and low scientific support.

In the 1960s and 1970s, implant-supported prostheses based on subperiosteal or blade implants had a poor reputation, mainly because of questionable clinical outcomes and lack of scientific documentation, encouraging the transformation into a scientifically sound discipline initiated by the two scientific pioneers of modern implant dentistry, Professor P. I. Brånemark from the University of Gothenburg in Sweden and Professor André Schroeder from the University of Bern in Switzerland. Together with their teams, and independently of each other, they laid the foundation for the most significant development and paradigm shift in dental medicine.

P.I. Brånemark was an experienced orthopedist studying bone physiology with titanium microcameras in the bone of several dogs when he found that the cameras had remained osseointegrated.(Brånemark et al. 1977)

Focussing on this phenomena Brånemark and his team decided to lay down the foundations of the first comprehensive study of the integration of titanium to bone and its application in oral rehabilitation. (T Albrektsson et al. 1981a; Adell et al. 1981b; Brånemark et al. 1983)

Together with his team, Adele, Jemt and others, they brought out the first

publication on osseointegration of dental implants in 1977 (Brånemark et al. 1977) opening the way to a new era.

Originally direct bone-to-implant contact (i.e. osseointegration) was referred to as direct bone deposition on the implant surface without interposition of fibrous or connective tissue (Brånemark et al. 1977), a term also called “functional ankylosis” (Schroeder et al. 1981) in contrast to the idea of Fibrointegration that was the accepted methodology at that time. (L. I. Linkow and Rinaldi 1987)

1.2. MODERN IMPLANTOLOGY

What came to be known as the post- Brånemark era, was based on a biological approach, the foundations of which can be found in the experimental methods reported by P.I. Brånemark who defined osseointegration as “the formation of a direct interface between an implant and bone, without intervening soft tissue”. (Brånemark 1983a)

One of the first definitions of survival and success was formulated by Albrektsson who stated that, for an implant to be successful over the years, it had to be free of infection, mobility and with marginal bone resorption of no more than 1,5 mm in the first year and 0,1mm in the following years. (T Albrektsson et al. 1981b)

In 1982 the Toronto Osseointegration Conference in Clinical Dentistry was held, introducing and validating the concept of osseointegration. The Toronto conference would provide a springboard for dental implantology out of an era of unpredictable and often short-lived treatment outcomes based upon limited research, to an evidence-based and predictable procedure, providing long-term replacement of failing and missing teeth. (Norkin 2012).

Implant dentistry was mainly being performed in Europe to treat fully edentulous patients, but with the Toronto conference, Professor George A. Zarb of the Faculty of Dentistry in Toronto introduced the concept of osseointegration, along with its application in treating edentulous patients in North America. (Zarb and Symington 1983).

In 1985, Nobelpharma AB Sweden (today Nobel Biocare, Switzerland) filed the

first application for commercial use of dental implants in the United States and in 1986 the American Academy of Implant Dentistry, which fostered the advancement of the field, was created.

1.3. HARD TISSUE INTEGRATION EVENTS

For osseointegration to occur when a dental implant is inserted a number of physiological and biochemical events take place.

Osseointegration is considered a healing process of bone in reaction to an alloplastic material in a foreign body reaction type. (van Steenberghe 1988; Brånemark et al. 1983)

Titanium is usually the material of choice but zirconia (Pieralli et al. 2017) and gold (Ingemar Abrahamsson and Cardaropoli 2007) have also proven osseointegration qualities.

Just a few minutes following implant installation, a blood clot is formed around the passivated titanium shell, produced by air contact from the implant surface to oxygen (passivation layer). (John E Davies 2003)

In rough surface implants (implants that undergo surface treatment after milling) the phenomena is called “contact osteogenesis” (J E Davies 2017), meaning that the first response of the clot is to adhere to the implant wall, in contrast to “distant osteogenesis” a characteristic of the “machined implants”. (the clot contracts first, and only a few hours later the implant wall is found).

After a blood clot is formed in the first 48 hours, inflammatory cells and biochemically induced proteins are released into the environment.

The auto immune response starts to release unspecific components to the area, mainly in the form of macrophages, neutrophils and lymphocytes.

Macrophages have a key function in wound healing and presumably also in bone regeneration, since the regulatory release of interleukins and other cell mediators are first released by these molecules.

After the first week, granulation tissue is formed and numerous blood capillaries that are embedded in the loose connective tissue are produced by fibroblasts.

Woven bone is a rapidly (approximately 10 µm/day) growing mineralized tissue characterized by means of haphazardly oriented collagen fibers and many large osteocyte lacunae.

Lamellar bone forms at approximately 1-2 µm/day, also on the surface of previously formed woven bone. (Shah et al. 2014; Brånemark 1983b)

Bone remodeling, which is a synonym for bone turnover, causes a mineralized structure of exclusively lamellar bone, thus creating a sound and hard tissue around the titanium surface.

1.4. OSTEOGENESIS

Skeletal basal bone formation may have two different origins: the direct conversion of mesenchymal tissue into bone, is called intramembranous ossification and the process by which a cartilage intermediate is formed and replaced by bone cells, is called endochondral (they are formed through apposition or as part of an endochondral matrix). (Dirckx, Van Hul, and Maes 2013)

The upper maxilla has an endochondral formation, on the first branchial arch. Endochondral ossification involves the formation of cartilage tissue from aggregated mesenchymal cells, and the subsequent replacement of cartilage tissue by bone.

The mandible has an intramembranous aetiology. Intramembranous ossification is the characteristic way in which the flat bones of the skull mineralize. (Ornitz and Marie 2015)

1.5. TOOTH FORMATION AND PERIODONTAL BONE FORMATION

The basic steps of tooth morphogenesis were well described over 100 years ago and are basically similar in all vertebrates.

The establishment of the dental lamina, the area that forms teeth, precedes the initiation of individual teeth. Teeth become visible during the following stages of

development, called the bud and the cap stages, with the appearance of the initial epithelial invagination and the tooth crown area, respectively. The cap stage is followed by the bell stage, during which species-specific cusp patterns emerge. (Soukup et al. 2008)

After the formation of the cusp pattern, the tooth grows to its final size, and mesenchymal odontoblasts and epithelial ameloblasts differentiate at the epithelial-mesenchymal interface to form dentin and enamel, respectively. These hard-dental tissues, together with cementum, which is made by cementoblasts, have largely similar compositions in all vertebrates, with enamel comprising up to 98% hydroxyapatite.

Periodontal bone (alveolar crest/bundle bone/lamina dura) came from the ectomesenquimal cells that form the dental papilla, precursor of the tooth germen and the periodontal tissues. (Jernvall and Thesleff 2012)

The formation of periodontal bone is different from the formation of endochondral or intramembranous bone. In terms of the formation of the Hertzwig sheath, there is a biochemical and molecular signalling interaction, inducing the creation of a row of osteoblasts and cementoblasts that create structures capable of achieving a true adhesion by means of the Sharpey fibbers. Thus, on the side of the tooth we have cement and on the side of the bone, a cortical structure called the bone crest, lamina dura, alveolar or bundle bone.

The interaction of these tissues is of utmost importance since the regulation in health or in pathology is made by means of several biochemical signs.

Interleukins are of critical importance to the cortical remodelling of this periodontal bone. Released by inflammatory mechanisms through macrophages and neutrophils IL-1β and IL6, they are able to determine the loss of insertion and bone remodelling, epithelial transformation factors such as IGF and FTβ released by fibroblasts which also serve as regulatory mechanisms.

The importance of this tooth-alveolar crest interaction is so critical that the extraction of teeth induces a remodelling pattern that eventually leads to bone

resorption and deformity of the area. (Araujo and Lindhe 2005).

1.6. OSTEOBLAST

The skeletal structure is constantly renewed by the balance osteoblast (responsible for laying down bone) and osteoclast (responsible for bone resorption).

The osteoblasts secrete a collagen-proteoglycan matrix that can bind calcium salts. Through this binding, the prebone (osteoid) matrix becomes calcified. In most cases, osteoblasts are separated from the region of calcification by a layer of the osteoid matrix they secrete. Occasionally, though, osteoblasts become trapped in the calcified matrix and become osteocytes—bone cells. As calcification proceeds, bony spicules radiate out from the region where ossification began. (Dirckx, Van Hul, and Maes 2013).

1.7. OSTEOCLAST

Destruction of bone tissue is due to osteoclasts, multinucleated cells that enter the bone through the blood vessels (Kahn and Simmons 1975; Manolagas and Jilka 1995). Osteoclasts are probably derived from the same precursors as macrophage blood cells, and they dissolve both the inorganic and the protein portions of the bone matrix (Blair et al. 1986). Each osteoclast extends numerous cellular processes into the matrix and pumps out hydrogen ions onto the surrounding material, thereby acidifying and solubilizing it.

In osteoclast differentiation, hormones regulate production and the hormonal changes of aging and may cause osteoporosis by increasing the number of osteoclasts. The conversion of a macrophage stem cell into an osteoclast is regulated by osteoprotegerin and its ligand. It is through these,s that signals on osteoblasts instruct the progenitor cell to become an osteoclast. (Boyce 2013).

Osteoclasts resorb bone and are responsible for numerous pathologies in relation to bone tissue, but also play a key role and factors in a number of bone metastases. (Ishii and Kikuta 2013).

They are a key target for oncology drugs such as bisphosphonates and other antiresorptive drugs. (Pelaz et al. 2015)

Osteoclasts are mainly responsible for marginal bone loss on dental implants and are key to any discussion of long term survival rate and dental implant complications. (Bang et al. 2014)

A number of implant researchers are including bisphosphonate in implant surfaces to try to decrease bone resorption around dental implants and thus secure a more stable complex. (Pyo et al. 2017)

Several investigations have tried to control the role of the interleukins on osteoclast activation on a biochemical level. We know that interleukin 1β and interleukin 6 in particular are potent chemical activators of these cell lineages. (Farhat et al. 2017a)

1.8. BONE BIOLOGY AND PHENOTYPES

Lekholm and Zarb maintain the classification system of bone as follows: Bone quality has been classified in four categories based on radiographic appearance and the resistance to drilling: Type 1 bone, in which almost the entire bone is composed of homogenous compact bone; Type 2 bone, in which a thick layer of compact bone surrounds a core of dense trabecular bone; Type 3 bone, in which a thin layer of cortical bone surrounds a core of dense trabecular bone; and Type 4 bone characterized as a thin layer of cortical bone surrounding a core of low density trabecular bone of poor strength. (Brånemark et al. 1983)

These differences in bone quality can be associated with different areas of anatomy in the upper and lower jaw. Mandibles are generally more densely corticated than maxillae and both jaws tend to show a decrease in their cortical thickness and an increase in their trabecular porosity as they move posteriorly. It has been shown, although there are have been counter studies, that there is a decrease in success rates as the bone type increases. (Cobo-Vazquez et al. 2017)

There have been a range of statistics on implant survival that have been reported according to bone quality, from a 2% difference from type 1 (98% in 36 months) to type 4 (96% in 36 months) to a 14% difference in another group (90% type 1 vs. 76% type 4 in 36 months). (Marquezan et al. 2012)

These are important statistics, as this indicates that bone quality is significant when considering an implant placement site, and secondly there appear to be other factors in the success rates of implants as one considers the vast discrepancy between the results. (Shadid, Sadaqah, and Othman 2014)

1.9. CLINICAL FACTORS IN IMPLANT DENTISTRY

1.9.1. Primary Stability

Osseointegration or secondary stability is the ability of an implant to be integrated into the surrounding bone structures. Secondary stability offers biological stability through bone regeneration and remodeling.

Primary stability mostly occurs from mechanical attachment with cortical bone and is affected by bone quality and quantity, surgical technique and implant geometry (length, diameter, surface characteristics). (Turkyilmaz and McGlumphy 2008)

Secondary stability is affected in part by primary stability but not entirely, since osseointegration is a multifactorial equation where stability is only one factor. (Esposito et al. 2013)

1.9.2. Stability Measurements

Invasive/destructive methods

The following methods are reported in the literature (the most important ones):

- Histologic/histomorphologic analysis
- Tensional test
- Push-out/pull-out test and
- Removal torque analysis.

Histomorphometric analysis

This is obtained by calculating the peri-implant bone quantity and bone-implant contact (BIC) from a dyed specimen of the implant and peri-implant bone.

Tensional test

The Tensional test is measured by detaching the implant plate from the supporting bone. It was later modified by Bränemark by applying the lateral load to the implant fixture.

Push-out/pull-out test

Push-out/pull-out test investigates the healing capabilities at the bone implant interface. It measures interfacial shear strength by applying load parallel to the implant-bone interface. The typical push-out or pull-out test checks tensile or compressive stresses. (Brunski *et al*. 2000, Chang *et al*. 2010).

There are Noninvasive/nondestructive methods for assessing implant stability including:

- Surgeon's point of view
- Radiographical analysis/imaging techniques
- Insertion torque measurement
- Reverse torque
- Seating torque test
- Percussion test
- Periotest
- Resonance frequency analysis (RFA): Electronic technology
- Magnetic technology.

Point of view of the Surgeon

One method of trying to evaluate primary stability is quite simply the perception of the surgeon. This is often based on the cutting resistance and seating torque of the implant during insertion.

1.9.3. Insertion torque measurement

Insertion torque values have been used to measure the bone quality in various parts of the jaw during implant placement. Insertion torque alone may be used as an independent stability measurement, but it may also act as a variable, affecting implant stability. In a different way, insertion torque is a mechanical parameter generally affected by a surgical procedure, implant design and bone quality at the implant site. However, it cannot assess the secondary stability by new bone formation and remodeling around the implant. Hence, it cannot collect longitudinal data to assess implant stability change after placement. Furthermore, an increase in insertion torque may lead to an increase in primary stability, but maximum insertion torque is produced by the pressure of the implant neck on the dense cortical bone of the alveolus. Furthermore, it has been reported that maximum insertion torque does not mean increased general bone density, it may indicate the insertion torque itself during tapping.

1.9.4. Percussion test

A percussion test is one of the simplest methods that can be used to estimate the level of osseointegration. This test is based upon vibrational-acoustic science and impact response theory. The clinical judgment on osseointegration is based on the sound heard upon percussion with a metallic instrument. A clearly ringing “crystal” sound indicates successful osseointegration, whereas a “dull” sound may indicate “no osseointegration”. However, this method relies heavily on the clinician's experience level and subjective belief. Therefore, it cannot be used experimentally as a standardized testing method.

1.9.5. Periotest

This test quantifies the mobility of an implant by measuring the reaction of the peri-implant tissues to a defined impact load. The Periotest® was introduced by Schulte to perform measurements of the damping characteristics of the periodontal ligament, thus assessing the mobility of natural tooth. Periotest® uses an electro-magnetically driven and electronically controlled tapping metallic rod in a handpiece. Periotest® value range from −8 (low mobility) to +50 (high mobility). It can measure the bone density at the time of implant placement and postsurgical placement of the implant. Response to a striking or “barking” is measured by a small accelerometer attached to the head. The reliability of this method is questionable because of poor sensitivity and susceptibility to many variables.

1.9.6. Resonance frequency analysis RFA

RFA was put forward by Meredith in 1998. It is a noninvasive diagnostic method that measures implant stability and bone density at various points in time using vibration and a principle of structural analysis. RFA utilizes a small L-shaped transducer that is tightened to the implant or abutment by a screw. The transducer comprises two ceramic elements, one of which is vibrated by a sinusoidal signal (5–15 kHz) while the other serves as a receptor. The transducer is screwed directly to the implant body and shakes the implant at a constant input and amplitude, starting at a low frequency and increasing in pitch until the implant resonates. High frequency resonance indicates stronger bone-implant interface. It also provides baseline reading for future comparison and postsurgical placement of the implant. RFA has been widely used for clinically assessing osseointegration, as well as for prognostic evaluation. However, the latter aspect still has to be questioned.

The most recent version of RFA is a wireless, where a metal rod is attached to the implant with a screw connection. The rod has a small magnet attached to its top that is stimulated by magnetic impulses from a handheld electronic device. The rod mounted on the implant has two fundamental resonance frequencies; it

vibrates in two directions, perpendicular to each other. One of the vibrations is in the direction where the implant is most stable and the other is in the direction where the implant is least stable.

Currently, two RFA machines are in clinical use: Osstell® (integration diagnostics) and Implomates® (Bio TechOne).

RFA was the first commercially available product for measuring implant stability. The electronic technology combines the transducer, computerized analysis and the excitation source into one machine which measures the implant stability quotient (ISQ of 0 to 100). When used at the time of implant placement it provides baseline reading for future comparison and postsurgical placement of the implant. Currently, Osstell (Integration Diagnostic AB, Goteborg, Sweden), a commercial product utilizing the concept of RFA has translated the resonance frequency ranging from 3000 to 8500 Hz as corresponding to an ISQ of 0–100

There are many clinical studies which correlate primary stability and Osstell values.

Lages studied the impact of abutment height on primary stability and concluded that it has a significant impact on resonance frequency analysis measurements. The higher the transmucosal abutment height, the lower the implant stability quotient value (Lages et al. 2017). To find a correlate between trabecular bone and cortical bone a study showed that there is a positive association between implant primary stability and bone mineral density at the receptor site. (Marquezan et al. 2012)

Aksoy undertook a study comparing Hounsfield units (HU) and Osstell values and the results demonstrated that computerized tomography measurements in terms of HU evaluations may be a helpful technique for predicting primary stability of the implant and bone quality (Aksoy, Eratalay, and Tözüm 2009). Bergkvist also did research on the same topic and also concluded that computed tomographic examination can be used as a preoperative method to assess jawbone density before implant placement, since density values correlate with prevailing methods of measuring implant stability. In his closing remarks he also stated that there were no differences in survival rates or changes in marginal bone level between implants placed in bone tissue of

different density (Bergkvist et al. 2017). Farre did a similar study proving the same concept, finding a strong relationship between bone density values, from computerized tomography and the location of the maxillaries. (Farré-Pagés et al. 2011) (Pagliani et al. 2012a)

It can be concluded from these studies that a correlation exists between bone quality according to the Lekholm & Zarb classification, and HU computerized tomography values. The primary implant stability measured with resonance frequency analysis depends on bone density values, bone quality and implant location.

To explore the relationship between primary implant stability and different parameters related to implant or bone properties Merheb did an in vitro study and concluded that Implant length, diameter or the presence of bony dehiscence did not have a significant effect on the mean RFA scores at implant insertion. (Merheb et al. 2010)

In a study to determine local bone density in dental implant recipient sites, using computerized tomography (CT), and to investigate the influence of local bone density on implant stability parameters and implant success, the mean bone density, insertion torque and RFA values were read.

The results were 645 ± 240 HU, 37.2 ± 7 N/cm^2, and 67.1 ± 7 ISQ for 280 successful implants at implant placement, while corresponding values were 267 ± 47 HU, 21.8 ± 4 N/cm^2, and 46.5 ± 4 ISQ for 20 failed implants. This indicated statistically significant differences for each parameter. (Turkyilmaz and McGlumphy 2008)

Waechter et al. did a study comparing clinical stability outcomes of tapered and cylindrical implants, comparing the clinical outcomes and studying their effect on bone site characteristics and peri-implant health during healing.

The author concluded that tapered and cylindrical implants show similar biological behavior during the healing process. Bone site characteristics are more important factors that can influence insertion torque and implant stability. (Waechter et al. 2017)

A similar study was undertaken by Gehrke to assess implant stability in relation to implant design (conical vs. semi-conical and wide-pitch vs narrow-pitch)

using resonance frequency analysis.

In this study, which is similar to our thesis, implant stability quotient (ISQ) was measured by resonance frequency analysis immediately following implant placement to assess primary stability (time 1) and at 90 days after placement (time 2). The results showed the mean and standard deviation ISQ for time 1 was 65.8 ± 6.22 (95% confidence interval [CI], 55 to 80), and 68.0 ± 5.52 (95% CI, 57 to 77) for time 2.

The results go against the findings of Waechter and in this study the author concluded that the greater primary stability of conical implants with wide pitch compared to semi-conical implants with narrow pitch might suggest a preference for the former in case of the adoption of immediate or early loading protocols. (Gehrke, da Silva, and Del Fabbro 2015)

1.10. MECHANICAL FACTORS IN DENTAL IMPLANTS

1.10.1. Macrogeometry

“The connection" is the link between the implant and the prosthesis and can be external or internal.

"The neck" is the part of the implant that emerges from the bone and is engaged with the implant soft tissue attachment.

“The body" is a part of the implant that is in contact with the bone and becomes osseointegrated.

There have been controversies surrounding implant body design (IBD). Implant body type can be categorized into 4 classes based on the shape: straight (ex: Brånemark), tapered (ex: 3INT, Replace), progressive tapered (ex: Ankylos) and dome type (ex: Biolok, Bicon).

P.I. Brånemark et al. reported “Osseointegrated implants in the treatment of the edentulous jaw” using a parallel sided dental implant with high survival rate (81% of the maxillary and 91% of the mandible) in 1977 and 1981(Adell et al. 1981c; Brånemark et al. 1977) This led to a number of implant manufacturers following the straight implant design. There have been many long-term studies

regarding success of straight implants. However, these implants showed a lower survival rate in soft bone (Fugazzotto, Wheeler, and Lindsay 1993). Engquist and Jaffin et al. reported that survival rate of straight type implant dropped to low of 65% in the soft bone in the maxillary posterior (B Engquist et al. 1988)(Jaffin and Berman 1991). While some may relate this to IBD, studies have shown that this lower rate may be attributed to the machined surface implants (Esposito et al. 2015), used in the Friberg study.

1.10.2. Tapered dental implant

The tapered dental implant was initially designed for immediate implant placement after tooth extraction. Today this implant is also used in cases where anatomical limitations prevent implant placement in an ideal restorative position. The alveolar process in the maxilla, particularly in the anterior and bicuspid regions, often displays pronounced buccal concavities. The use of tapered implants allows for more axial positioning with less chance of apical fenestration of the alveolar process. Tapered implants are also useful in the mandibular posterior region, where there is a lingual concavity due to the presence of the mandibular gland fossa. Other clinical situations, where tapered implants are useful are when there are converging roots of teeth adjacent to the implant site, sinus medial or distal wall proximity and when employing a ridge splitting technique. (Shapoff 2002).

Tapered implant placement shows that a higher apical torque bone compression occurs during the last revolution of placement, increasing implant rigidity and stability. O'Sullivan et al. reported that 1 degree of taper results in better primary stability compared with the standard Brånemark design (O'Sullivan, Sennerby, and Meredith 2000). In an animal study, they measured initial stability using insertion torque (IT), resonance frequency analysis (RFA) and removal torque (RT) at the time of insertion. However, 6 weeks postoperatively when the animals were sacrificed, there was no significant difference in secondary stability. Friberg et al. also reported that tapered implants more frequently required a higher insertion torque and showed a significantly higher primary stability than straight type implants in the soft bone.

He recommended tapered implants when placing implants in jaw regions of type 4 bone and showed a higher success rates (test implant side; 93.1%, control implant side; 88.4%) (Friberg, Jemt, and Lekholm 1991).

1.10.3. Progressive thread dental implant

The thread depth increases towards the apex with a “progressive thread implant”. The manufacturer claims that this thread design distributes the chewing forces toward the flexible spongy bone while providing simultaneous load relief at the cervical region. The rationale for this design is that the relatively elastic spongy bone, which contacts about 90% of the implant body, decreases in volume in the cervical direction and becomes less elastic because of the cortical supporting shell, whose rigidity is approximately 10 times higher than the spongy bone (Nentwig 2004). However, to date there have been no controlled comparative studies, indicating that this IBD or thread design results in a higher implant survival rate.

Other factors such as thread design, surface roughness, length and diameter of the implant fixture have all been shown to influence survival rate of dental implants. The importance of these variables related to that of IBD remains to be determined in controlled clinical trials.

1.10.4. Implant Platform

The main goal of all implant manufacturers is to achieve a strong and durable connection between the implant and the restorative component which can be accurately positioned and reproduced when abutment parts are removed and replaced, and which maintain a precise position. There are two types of connections, the external and the internal (Finger et al. 2003) connection. The External, has been the most commonly used connection in a "hexagonal" configuration. The purpose of this geometry was to achieve an anti-rotational mechanical effect allowing reproducibility of the restorative component. This connection has several disadvantages, including a small engagement length a

rotational "slop or wobble" on the hex and a strain on the connecting screws. The slop or wobble leads to a rotation of the abutment on the hex, because there is a space between the mating parts that allows for the seating of the prosthetic component on the implant (Carr et al. 2017). Other connection designs include the "External Spline and Octagon". The external octagon is a one-piece narrow diameter implant particularly designed for mandibular anterior use (ITI Narrow Neck), characterized by a tall octagon, which allows 45-degree rotation, good lateral and rotational resistance and good strength.

Gracis in 2012 showed that the major prosthodontic complication on the external hex was screw loosening 3.0% compared to only 2.1% in the internal connection, 7,5 % with a metal abutment. (Gracis et al. 2012)

If we go to the gold standard of evidence in the literature - the randomized clinical human trials and the systematic reviews - we see that Schwartz et al. (Schwarz, Hegewald, and Becker 2014) failed to answer the question of which connections have the higher survival rate, Esposito et al. in a RCT of 102 patients with 173 external hexagon implants and 98 patients with 154 internal connection implants, found no statistical significant differences between the two connection types. (Esposito et al. 2017).

Medeiros et al. undertook a systematic review of external hex versus internal and found a slight advantage for the use of an internal connection when we use platform switch implants. When comparing dental implants with internal connections research revealed lower marginal bone loss than implants with external connections. This finding is mainly the result of the platform switching concept, which is more frequently found with internal connections. (de Medeiros et al. 2016)

Pessoa et al. found that the marginal bone loss was significantly different between internal and external, favoring the internal. But no significant microbiological and clinical differences could be observed. (Pessoa et al. 2017)

The internal connection is designed with the abutment and the screw of the restorative component sliding down inside the implant body. The deeper the insertion of the abutment, the more the engagement length is increased, and with less strain on the retaining screw. Because of the extreme force developed

by this system, obviously the implant body wall needs to have sufficient bulk and strength (Carr et al. 2017). There are two different internal connections available in the market. They include the "morse taper" and the "butt joint".

1.11. SUCCESS AND SURVIVAL DEFINITION IN DENTAL IMPLANTS

In 1986, Albrektsson et al. established the following criteria for implant success: the implant should have no mobility and demonstrate no radiolucent areas, radiographically. In the first year marginal bone remodeling should be in the range of 1 to 2 mm and annual vertical bone loss after the first year less than 0.2 mm, and there should be no persistent and/or irreversible symptoms. (T Albrektsson et al. 1986).

Although the above is the most quoted criteria, there are several other publications that have defined success in oral implantology. In 1979 Schnitman et al. defined implant success as being mobility less than 1 mm in any direction, bone loss no greater than one third of the vertical height of the bone, gingival inflammation amenable to treatment, absence of symptoms and infection, absence of damage to adjacent teeth, absence of paresthesia and anesthesia or violation of the mandibular canal, maxillary sinus or floor of the nasal passage, functional service for 5 years in 75% of patients. (Schnitman and Shulman 1979)

In addition, Cranin et al. maintained in 1982 that success was defined as the implant remaining in place for 60 months or more, lack of significant evidence of cervical saucerisation on radiographs, free from hemorrhage, lack of mobility, absence of pain or percussive tenderness, no pericervical granulomatosis or gingival hyperplasia and no evidence of a widening peri-implant space on radiograph. (Laney and Chairman 1990)

Although the definition of success of dental implants is always being updated, the most common parameter used in clinical reports is survival rate, indicating if the dental implant is physically in the mouth or has been removed.

One of the problems in the literature is that implants are reported in terms of their survival status and not on their success rate, meaning that they are only

reported if the implant is functioning, without any information on their biological state. The term survival can therefore be a very dangerous and misleading source of information for the reader.

The term success however, has been constantly changing since the postulates of Thomas Albrektson, with the development of new surfaces and connections and with new surgical protocols where the initial 1,5 mm of bone remodeling may not be true for all implants as is the figure of 0,2 mm per year.

New parameters such as esthetics and patient satisfaction were added to the definition of success rate in oral implant dentistry.

Success can also be measured according to peri-implant measurements such as: visible plaque index (VPI), marginal bleeding index (MBI), probing depth (PD), bleeding on probing (BOP) and clinical attachment level (CAL). (Nicoli et al. 2017)

The width of the attached gingiva, co-existing medical conditions, smoking, and width of the implant also play a role in evaluating implant success. Genetic and immunological markers have also been identified. (Karthik et al. 2013).

Today in 2018, the criteria for success have to merge esthetic, functional and radiographic criteria and is implant dependent. The cumulative survival rates accepted in the literature are on average 98.0% for patients and 98.7% for implants after 6 years of observation time (Lin et al. 2018). In addition, the clinical parameters, particularly probing depth, might accurately locate diagnoses among peri-implant conditions. (Monje et al. 2018)

1.12. BIOLOGICAL WIDTH FORMATION ON TEETH AND DENTAL IMPLANTS

The formation of the biological width is a mechanism common to the entire human species and basically corresponds to the ability of the human body to cover exposed bone with periosteum and connective tissue, and this layer with the ability to coat itself with epithelium.

In the oral cavity and more specifically in the periodontal area, the marginal bone / alveolar bone can protect itself with these two layers of connective tissue and epithelium.

In the internal part of the periodontal complex lies the periodontal sulcus, composed of: sulcular epithelium (an area of epithelial adhesion to the enamel of the tooth), the junctional epithelium and below this , the connective tissue that supports all the supracrestal fibers which along with other functions, promotes marginal periodontal integrity through the adhesion of collagen fibers to the cementum of the tooth root. (Gargiulo, Wentz, and Orban 1961).

The distance from the most coronal part of the epithelium of the junctional epithelium (of which the sulcular epithelium is not part) to the most apical zone of the connective tissue (usually equivalent to a line perpendicular to the most coronal point of the marginal bone crest) is known as periodontal biological width (BW)). (Nugala et al. 2012)

In terms of peri-implant tissues this is similar to the biological width formation on teeth. The same layers are present (periosteum, connective tissue and epithelium) but with slightly different features, the most important of which is the formation of the BW subcrestal contrasting to the supracrestal formation on teeth. (I Abrahamsson, Berglundh, and Lindhe 1997)

There are several works in the literature that classify and measure the corresponding height of BW present in periodontal and peri-implant tissues. Recently some works attempted to validate the work of Gargiulo et al., but findings showed that the BW is statistically significantly lower than the value stated by Gargiulo et al. (2,04 mm) with a mean value of 1,13 mm, whereas the SD is statistically significantly greater than the value stated by Gargiulo et al. (0,69 mm) with a mean value of 1,96 mm according to one of the most recent systematic reviews on the subject. (Hamasni and El Hajj 2018)

In relation to the BW in the peri-implant area, a number of works have established a correlation between several approaches and subsequent marginal bone loss.

In the peri-implant tissues, Researchers attempted to correlate biological width formation and marginal bone loss. Judjar et al. in a human study found a mean 3.26 ± 0.15 mm BW in implants placed subcrestally. (Judgar et al. 2014).

The results were between an interval of 2 and 4 mm of BW formation in the animal subject, particularly in the dog model, Negri el al. found a mean average

of 3,34 ± 0,53 mm (Negri et al. 2015), Huh et al. found the same values averaging 2,88 ± 0,66 mm to 3,18 ± 0,63 mm when comparing the BW to different surfaces (Huh et al. 2014), Cochran et al. tried to establish the BW formation on implants in different positions- the infracrestal, equicrestal and supra crestal and he found a mean average of 2,33 ± 0,729 mm to 1,77 ± 0,340 mm supporting the supracrestal aproach (Cochran et al. 2017). Linares et al. tried to modify the collar of the implant in the minipig model in order to see if there was any influence in terms of the biomaterial on the BW formation, and found an average of 2,12 ± 0,35 mm for the machined collar and 2,14 ± 0,46 mm for the zirconia metal collar indicating that there is no statistical difference for the BW formation. (Liñares et al. 2015)

in 2012 Blanco et al. began a series of articles related to the BW formation in different scenarios. In the dog model he found no statistical differences between flap vs flapless approach resulting in a BW of 4,01 ± 0,64 mm and 3,9 ± 0,64 mm. (Blanco et al. 2012).

In the same dog model Blanco observed BW formation on rough surface vs polished collar and found an average 3,69 mm (Blanco et al. 2010). Another study from the same team on the BW formation in immediate loading cases showed a BW formation on average of 3,20 mm (Mareque et al. 2014). The last article from the BW series was published in 2016 and again showed an average BW of around 3,01 ± 0,44 mm (Blanco et al. 2016a). This last study has the curiosity of being very similar in purpose to this PhD - our study measured the impact of having zirconia, acrylic and titanium on the molecular signalling, while in the 2016 study Blanco showed us the histomorphometric behaviour of zirconia and titanium with "*soft tissue dimension at Ti and ZrO2 similar in all counterparts: biological width, the length of the barrier epithelium, length of the connective tissue, and the percentage of collagen fiber density*". (Blanco et al. 2016a)

Finally, the study by Negri et al. in the dog model showed once again, the same average height of the biological width: 3,44 ± 0,47mm. (Negri et al. 2014)

In summarizing BW dimensions it seems that there may be a difference in the BW height between the animal model and the human model of about 1mm.

The human BW seems to be in the range of 1,92 to 3 mm while the animal model is between 2 and 4 mm.

1.13. MARGINAL BONE REMODELING/LOSS

In a systematic review of marginal bone loss Paul el al. (Paul, Petsch, and Held 2017) reported some highly useful results on the subject. The inclusion criteria and article selection only referred to RCT and human trials. In these inclusion criteria, we can see that MBL ranges from almost 0 to 3 mm depending on the implant surface, position and surgical technique employed.

If we break down the systematic review and analyse the individual articles we can extract more detail. For example, with the traditional external hexagon we see a tendency to obtain values around 1,5 to 2 mm. The average results of -1,89 ± 0,06 mm of MBL found by Engquist et al. backs this up of this (Bo Engquist et al. 2005). In addition, implants with rough surfaces seem to have less MBL, as shown in a number of studies: in Crespi et al. where the MBL on the acid-etch surface averaged -1,16 ± 0,51mm (Crespi et al. 2007),Cordaro on the SLA polished collar supracrestal who recorded an average of -0,54 ± 0,33 mm (Cordaro, Torsello, and Roccuzzo 2009) on the anodized surface the Turkish group of Cehreli et al. recorded an average of -1,21 ± 0,10 mm compared to internal SLA surface -0,73 ± 0,06 mm (Çehreli et al. 2010a). Another study of SLA surface, one stage implant placement, Enkling et al., recorded a mean average of MBL of 0.47 ± 0.46 mm (Enkling et al. 2011), Tallarico e al. with the Swiss group reported an average -0,87 ± 0,45 mm with an anodized surface. (Tallarico et al. 2016)

The Spanish group found an average of -0,68 ± 0,98 mm with the SLA (Sanz et al. 2015).The only study that showed an increase in bone gain was the study of Shibly reporting an average +0,75 ± 0,17 mm gain with the anodized surface (Shibly et al. 2012). Finally in the last three studies all appear to have a reduced MBL, when a one stage implant procedure is made with a supracrestal approach, - 0,16 ± 0,3 mm (Siadat et al. 2012a) -0,54 ± 0,76 mm (Cordaro et al. 2013) -0,249 ± 0,362 mm (Nader et al. 2016).

1.14. PLATFORM MATCHED VS PLATFORM SWITCH

In 1991, Implant Innovations Inc. (3i Palm beach garden, FL) introduced 5 mm. and 6 mm diameter implants. However, when these wider diameter implants were introduced, no matching dimensioned prosthetic components were available. As a result, clinicians had to restore them with standard 4.1 mm diameter abutments.

On reviewing radiographs of the patients where "Platform Switching" had been undertaken, the bone loss is normally attributed to the reformation of the biologic width (1.5mm) was not observed over a 5 year period. (Lazzara and Porter 2006)

Lazzara and Porter suggested that platform switching alters the inflammatory cell pathways, shifting them inward and away from the adjacent crestal bone and thereby limiting bone resorption around the implant. The crestal bone remodeling process which has been attributed by some authors to the implant being placed into function, is now considered a reaction of the peri-implant tissues to oral environment exposure (I Abrahamsson et al. 2002). Since biologic width formation begins immediately, it is important to understand that to benefit from "platform switching" the smaller diameter abutment component must be used from the moment the implant is uncovered or exposed to the oral cavity in either a one or two stage surgical approach. Utilizing platform switching at a later time is ineffective since the crestal bone will not return to its pre-surgical level following remodelling.

A plethora of articles on the subject were published following these, and in 2016/17 the first systematic reviews began to arrive. A meta-analysis undertaken by Strietzel revealed a significantly less mean MBL change with implants with a PS compared to PM-implant-abutment configuration, in 15 RCT the difference analyzed was 0,49 mm with platform switching to 1,3 mm with platform matching. (Strietzel, Neumann, and Hertel 2015a).

Hereaker, in a 2014 systematic review found, that marginal bone loss around platform-switched implants was significantly less than platform-matched implants (mean difference [MD]: -0,34; 95% confidence interval [CI]: -0,37 to -0,30; P < 0,001)(Herekar et al. 2014). Hsu evaluated 26 RCT studies involving

1,511 PS implants and 1,123 RP implants were evaluated where PS implants had a mean MBL of 0,36 ± 0,15 mm within the first year of use. (Hsu, Lin, and Wang 2017)

In others systematic reviews the conclusion generally favored platform switching over platform matching. (Monje and Pommer 2015) (Atieh, Ibrahim, and Atieh 2010)(Scapoli et al. 2012)

In one of the most quoted studies from a Portuguese group in Coimbra, concluded that in sixty-three patients with a total of 135 implants from surgery to 36 months, the mean bone loss was 0,28 ± 0,56 mm for the platform-switching group and 0,68 ± 0,64 mm for the platform-matching group. A statistically significant difference was found between groups (p = 0,002) with an estimate of 0,39 mm (0,15-0,64, 95% CI) in favor of platform. (Rocha et al. 2016)

Since in the highest levels of evidence, the literature favours PS, it seemed logical to choose this path for our study.

1.15. ONE ABUTMENT ONE TIME CONCEPT

Placing the final abutment on the day of surgery has its roots in the early works of Abrahamsson who maintained that the disconnections and subsequent reconnections of the abutment component of the implant compromised the mucosal barrier and resulted in a more "apically" positioned zone of connective tissue. The additional marginal bone resorption observed at the test sites following abutment manipulation may be the result of tissue reactions initiated to establish a proper "biological width" of the mucosal-implant barrier. (I Abrahamsson, Berglundh, and Lindhe 1997)

Iglhaut et al. in 2013 also reported this pattern in a human study. He found that repeated abutment dis-/reconnection during the initial healing phase (4-6 weeks) could be associated with increased soft- and hard-tissue changes and therefore a single abutment, done once approach should be considered. (Iglhaut et al. 2013)

Rodriguez studied the impact of one abutment placed once with the platform switch. He showed that Implants with a PS design, experience less peri-implant

bone resorption (over the healing process and as the abutments are disconnected) than comparably dis/reconnected platform-matched implants, (Rodríguez et al. 2013), again favouring the placement of the final abutment on the day of implant placement**.**

Finally, in a systematic review by Atieh, in a sample comprising a total of 1124 identified citations where seven trials with 363 dental implants in 262 participants were included in the analysis. The results favored the use of final abutments on the day of surgery. Atieh states clearly that there are favorable changes in peri-implant marginal bone level but he also qualifies this by saying that this technique should be viewed with caution as its clinical significance is still uncertain. (Atieh et al. 2017)

One abutment one time is a technique that literature supports with high–end literature.

In fact one of the most recent systematic reviews, with meta-analysis, revealed that implant restoration protocol using one-time abutments is superior to repeated abutment diconnection for platform switched implants measured in less bone resorption and soft tissue stability of the former. (Q.-Q. Wang et al. 2017)

1.16. PERIMPLANTITIS

The etiology of periodontal disease is, according to the literature, the presence of bacteria that will infect the peri-implant surface, jeopardizing implant survival and success.

Similar in nature to gingivitis and periodontitis affecting the periodontium of natural teeth, an inflammation and destruction of soft and hard tissues surrounding dental implants is known as mucositis or peri-implantitis. (Khammissa et al. 2012)

However, if we compare periodontitis to perimplantitis Carcuac et al. showed that in contrast to periodontitis samples, peri-implantitis lesions were more than twice as large and contained significant proportionately larger areas , numbers, and densities of CD138-, CD68-, and MPO-positive cells than periodontitis

lesions. (Carcuac and Berglundh 2014).

It is suggested that peri-implantitis is a common condition and that a number of patient and implant-related factors, influence the risk for moderate/severe peri-implantitis. Moderate/severe peri-implantitis (bleeding on probing/suppuration and bone loss >2 mm) was diagnosed in 14.5% of the Swedish population according to Derks. (Derks et al. 2016).

The problem with implant infections is that treatment is often very unpredictable according to a systematic review by Esposito. He clearly states that sample sizes were very small and follow-up too short, therefore the findings had to be considered with great caution (Esposito et al. 2010). Larger well-designed RCTs with a follow-up of longer than 1 year are needed. (Bottalico et al. 2017)(Alani and Bishop 2014)(Elemek and Almas 2014)(Figuero et al. 2014)(Russell et al. 2014)(Chan et al. 2014)

One review proposed a mixture of criteria for successful treatment outcome of perimplantitis, which consisted of implant survival with mean probing depth < 5 mm and no further bone loss. (Bottalico et al. 2017)

Successful treatment outcomes at 12 months were reported in 0% to 100% of patients treated in 9 studies and in 75% to 93% of implants treated in 2 studies they reported outcomes that must be viewed in the context of the varied peri-implantitis case definitions and severity of disease included, as well as the heterogeneity in study design, length of follow-up, and exclusion/inclusion criteria. (Heitz-Mayfield and Mombelli 2014)

The bottom line in this introductory topic on peri-implantitis is to show that the literature confirms that on the day of surgery, if we submerged the implant, the biological implication is that, bone will remain at implant level and the only remodelling that will exist, will be at the stage 2 abutment connection. The reason for this is that stage 2 will induce a microgap that will eventually become contaminated and through this provocation, bone tends to resorb and biological width will reform at an apical complex. (Jemt, Sundén Pikner, and Gröndahl 2015).

The theory of a sterilized microgap on the day of surgery backs up our research since we want to study the inflammatory reaction and the impact that this has

on MBL. Thus, if there is no bacteria on the day of surgery, the abutment is placed on the same day and all the measurements are made without taking the abutment on and off.

With regard to risk factors for perimplant disease, in a systematic review by Stacchi reveals that both implant and patient-based meta-analyses showed a significantly higher risk of developing peri-implantitis in patients with a history of periodontitis compared with periodontally healthy subjects. (Stacchi et al. 2016)

The literature favors this type of methodology. For example, a study by Scarano precisely confirms bacteria adherence to zirconia and states clearly that the results demonstrate that zirconium oxide is a suitable material for manufacturing implant abutments with a low colonization potential. (Scarano et al. 2004) (Rismanchian et al. 2012)(Scarano et al. 2016)(Heijdenrijk et al. 2006)(Smith and Turkyilmaz 2014)

With regard to the different materials and peri-implant disease, a systematic review by Esposito et al. maintained that in implant surface turned/machined surfaces there was a 20% reduction in risk of being affected by perimplantitis over a 3-year period. (Esposito et al. 2005).

Protein biomarkers showed increased levels of the selected PICF-derived biomarkers of periodontal tissue inflammation, matrix degradation/regulation, and alveolar bone turnover/resorption combined with site-specific microbial profiles possibly associated with the beginnings of peri-implantitis. (H.-L. Wang et al. 2016)

1.17. DENTAL IMPLANT INFLAMMATION

Autoimmune response

Autoimmune disease occurs when a specific adaptive immune response is mounted against self-antigens. The natural result of an adaptive immune response against a foreign antigen is the clearance of the antigen from the body.

Adaptive immune responses are initiated by the activation of antigen-specific T cells, and it is believed that autoimmunity is initiated in the same way. T-cell responses to self-antigens can inflict tissue damage either directly or indirectly.

In the case of placement of a dental implant there is an innate reaction of the organism to the titanium, zirconia and acrylic material. (Brooks 2012)

The question we wish to answer in this thesis is the extent to which there is a default host reaction against these alloplastic materials and if so, at what intensity this takes place.

Some expert opinions maintain that the inflammatory reaction may lead to greater marginal resorption, since it has been shown in “signaling biology” that the inflammatory response plays a crucial role. A late failure of osseointegration could be due to this autoimmune response.

1.18. THE ROLE OF INTERLEUKINS

1.18.1. Inflammation cascade

Inflammation is part of the complex biological response of body tissues to harmful stimuli, such as pathogens, damaged cells, or irritants,(Ferrero-Miliani et al. 2007) and is a protective response involving immune cells, blood vessels, and molecular mediators.

The function of inflammation is to eliminate the initial cause of cell injury, clear out necrotic cells and tissues damaged from the original trauma and the inflammatory process, and to initiate tissue repair.

1.18.2. Inflammation and dental implants

There is currently a list of 36 interleukins that have specific targets and functions in the human body. Although they are known to exist, only a part of them are well studied.

Interleukins are a group of cytokines (secreted proteins and signal molecules) that were first seen to be expressed by white blood cells (leukocytes).

Many parts of our body depend on these small molecules for regulation of function, but it is the immune system that depends mainly on interleukins.

Some genetic pathologies and rare disorders resulting from interleukin deficiencies have been described, all consistently featuring autoimmune diseases or immune deficiency. The majority of interleukins are synthesized by helper CD4 T lymphocytes, as well as through monocytes, macrophages, and endothelial cells. One special feature of interleukins is that they promote the development and differentiation of T and B lymphocytes and hematopoietic cells.

Interleukins intervene in almost all the regulatory processes of the human body. They are key signaling molecules that inform cells of the status of the body.

There are a number of interleukin receptors on cells. They can be found on heart cells, adipocytes and even on astrocytes in the hippocampus and are also known to be involved in the development of spatial memory in mice.

The name "interleukin" was chosen in 1979, to replace the various different names used by different research groups to designate interleukin 1 (lymphocyte activating factor, mitogenic protein, T-cell replacing factor III, B-cell activating factor, B-cell differentiation factor, and "Heidikine") and interleukin 2 (TSF, etc.).

The term interleukin derives from inter- "as a means of communication", and (-leukin) "deriving from the fact that many of these proteins are produced by leukocytes and act on leukocytes.

The most important and well-known cytokines that interfere with the oral cavity inflammation cascade are discussed below (IL2, IL8, IL-1β and IL6) but for our study we will focus in a more detailed way on IL-1β and IL6:

1.18.3. Interleukin-2 (IL-2)

An interleukin is a type of cytokine signaling molecule in the immune system. It is a protein that regulates the activities of white blood cells (leukocytes, often lymphocytes) that are responsible for immunity.

Interleukin-2 (IL-2) is a pleiotropic cytokine that drives T-cell growth, augments NK cytolytic activity, induces the differentiation of regulatory T cells, and mediates activation-induced cell death. Along with IL-4, IL-7, IL-9, IL-15, and IL-21, IL-2 shares the common cytokine receptor γ chain, γ(c), which is mutated in humans with X-linked severe combined immunodeficiency.

IL-2 is part of the body's natural response to microbial infection, and in discriminating between foreign ("non-self") and "self". IL-2 mediates its effects by binding to IL-2 receptors, which are expressed by lymphocytes. IL-2 plays essential roles in key functions of the immune system, tolerance and immunity, primarily via its direct effects on T cells, some evidence indicates that IL-2 is in some way involved in itchy psoriasis.

1.18.4. Interleukin-8 (IL-8)

IL-8, also known as neutrophil chemotactic factor, has two primary functions. It induces chemotaxis in target cells, primarily neutrophils but also other granulocytes, causing them to migrate toward the site of infection. IL-8 also induces phagocytosis once they have arrived. IL-8 is known to be a potent promoter of angiogenesis. In target cells, IL-8 induces a series of physiological responses required for migration and phagocytosis, such as increases in intracellular Ca^{2+}, exocytosis (e.g. histamine release), and the respiratory burst.

IL-8 can be secreted by any cells with toll-like receptors that are involved in the innate immune response. It is usually the macrophages that detect an antigen first, and thus are the first cells to release IL-8 to recruit other cells.

IL-8 is believed to play a role in the pathogenesis of bronchiolitis, a common respiratory tract disease caused by viral infection.

1.18.5. IL-1β

IL-1β is a member of the interleukin 1 family of cytokines. This cytokine is produced by activated macrophages as a proprotein, which is proteolytically

processed to its active form by caspase 1 (CASP1/ICE). This cytokine is an important mediator of the inflammatory response, and is involved in a variety of cellular activities, including cell proliferation, differentiation, and apoptosis. The induction of cyclooxygenase-2 (PTGS2/COX2) by this cytokine in the central nervous system (CNS) is found to contribute to inflammatory pain hypersensitivity. This gene and eight other interleukins 1 family genes form a cytokine gene cluster on chromosome 2.

1.18.6. IL6

Interleukins are a group of cytokines (secreted proteins and signal molecules) that were first seen to be expressed by white blood cells (leukocytes).(Farhat et al. 2017c)

The function of the immune system depends to a large part on interleukins, and rare disorders have been described, all in terms of autoimmune diseases or immune deficiency. (Farhat et al. 2017b)

Most interleukins are synthesized by helper CD4 T lymphocytes, as well as through monocytes, macrophages, and endothelial cells. They promote the development and differentiation of T and B lymphocytes, and hematopoietic cells. (Farhat et al. 2017c)

Interleukin-6 is a cytokine not only involved in inflammation and infection responses, but also in the regulation of metabolic, regenerative, and neural processes.

The human interferon-beta 2 gene (IFNB2) product is identical to that for the B-cell stimulation factor-2(BSF-2), the hybridoma growth factor(HGF) ("interleukin-6"), and the hepatocyte stimulating factor(HSF).

Cytokines of the IL6/GCSF/MGF family are glycoproteins of approximately 170 to 180 amino acid residues that contain four preserved cysteine residues involved in two disulphide bonds. They have a compact, globular fold (similar to other interleukins), stabilized by two disulphide bonds. One half of the structure is dominated by a 4-alpha-helix bundle with a left-handed twist; the helices are anti-parallel, with two overhand connections, which fall into a double-stranded

anti-parallel beta-sheet. The fourth alpha-helix is important to the biological activity of the molecule.

According to several authors, members of the interleukin 6 (IL6) family of cytokines include: IL6, IL-11, leukemia inhibitory factor (LIF), oncostatin M (OSM), ciliary inhibitory factor (CNTF), cardiotropin-1 (CT-1), cardiotrophin-like related cytokine and stimulating neurotrophin-1/B-cell stimulating factor 3 (NNT-1), neuropoietin (NPN), IL-27, and IL-31.

With the exception of IL-31, all IL6 type cytokines share the membrane glycoprotein gp130 as a common receptor and signal transducer subunit (reviewed in references 1 and 2).

There are several lines of evidence suggesting that IL6 plays a pivotal role during the transition from innate to acquired immunity. Acute inflammation is characterized by an initial infiltration of neutrophils, which is then replaced by monocytes and T cells after 24–48h to prevent increased tissue damage from the accumulation of neutrophil-secreted proteases and reactive oxygen-species at the site of inflammation.

This is exactly what occurs in the first hours of osseointegration. The lesion and trauma of endothelial cells as well as other vascular elements that are activated by trauma or microbial products, IL-1β or TNFα, produce various chemokines together with IL6, leading to the attraction of neutrophils in the initial phase of inflammation.

There are many known fields of action of the IL6. Besides its role in the recruitment and anti-apoptosis of T lymphocytes, it is also known that IL6 plays a crucial role in B and T cell differentiation. IL6 was initially characterized as a factor that enhances antibody production in a B cell line.

Proteins derived from this gene mediate the plasma protein response to tissue injury (acute-phase response) and regulate the growth and differentiation of both B and T cells. Interleukin-6 (IL6) has come to be regarded as a potential osteoporotic factor because it has stimulatory effects on cells of the osteoclast lineage, and, thus, may play a role in the pathogenesis of bone loss associated with estrogen deficiency. IL6 plays many roles essential to the regulation of the immune response, hematopoiesis, and bone resorption.

The most important activity of IL6 evolves bone homeostasis which is regulated by the balance of osteoblasts building up bone, and osteoclasts, which degrade bone. It has been shown that osteoclast formation is triggered by IL6 only in the presence of sIL-6R. The importance of IL6 was underlined by experiments with IL6 mice, which were completely protected from bone loss after ovariectomy, which is a model for bone loss in females after menopause.

As a result, it has been shown that neutralizing anti-IL6 mAbs inhibit osteoclast formation. Interestingly, IL6 levels and certain IL6 gene polymorphisms have been associated with bone mineral density alterations in inflammatory disease. Taken together, these data show that IL6, most likely via IL6 trans-signaling, plays an important role in the regulation of bone homeostasis.

1.18.7. IL-1β and IL6 in the oral cavity

Modern dentistry has put forward several bacteriological theories since the 90´s, in which all periodontal and tooth related pathologies were attributed to bacteria.

Recently, theories that correlated bacteria and dental/periodontal pathologies have failed to produce sufficient evidence and as a result, biochemically oriented theories started to gain ground.

The potential role of inflammation and auto-immune reaction to healthy tissue, thus arrived to open new doors of investigation.

Interleukins play a pivotal role in human pathologies from simple inflammation against virus or bacteria to metastatic cancer regulation and other genetic diseases.

1.18.8. Interleukin influence on periodontal health and disease

Interleukins and periodontal disease are well documented, although sometimes with opposing results.

In the periodontal disease literature, the role of interleukins has always been

linked to pathologic states. One recent study with 330 individuals (134 cases, 196 controls) were genotyped for the IL6 by PCR technique. The results showed increased levels of salivary IL6 in periodontitis patients and concluded that IL6 may be considered as an important marker for periodontitis. (Gabriela Teixeira et al. 2014)

Another genetic study for periodontal disease reported that the gene portion rs1800795 SNP located in the IL6 gene promoter, was strongly associated with the occurrence of both gingivitis and periodontitis. Indeed, in the same study homozygous individuals with variant allele appeared less-susceptible to both gingivitis OR=0.47 (95% C.I. 0.27-0.82) and periodontitis OR=0.36 (95% C.I. 0.21-0.64). This data confirmed the role of IL6 in susceptibility to periodontitis among the Italian population. (Scapoli et al. 2012)

In epidemiologic studies, Scapoli et al. organized a cohort of 184 patients with chronic periodontitis and 231 healthy controls. A total of six single nucleotide polymorphisms from five candidate genes, i.e., IL1α, IL-1β, IL6, IL10 and vitamin D receptor, were investigated. The rarer variant allele lowered the risk of developing periodontitis at IL6 (Odds Ratio [OR] = 0.69 [95% confidence interval {CI} 0.51-0.93]) and increased the risk at IL10 (OR = 1.38 [95% CI 1.01-1.86]). This indicated, once again, that polymorphisms of IL6 and IL10 constitute risk factors for chronic periodontitis. (Scapoli et al. 2012)

IL-1β has also been studied in the periodontal literature and a study by an Iranian group investigated the association of a variable number of tandem repeat polymorphism in the IL1RN gene with generalized aggressive periodontitis.

Their findings suggest that the polymorphic IL-1β gene is a risk determinant for generalized aggressive periodontitis in the Iranian Khorasanian population. (Baradaran-Rahimi et al. 2010)

Again, in the IL-1β literature, the aim of the Karanesh study, was to investigate if IL-1 gene cluster polymorphisms are associated with chronic (CP) and aggressive (AgP) periodontitis in a Jordanian population. The conclusion was that IL-1RN 8006 and IL-1RN VNTR were associated with CP but not AgP in the Jordanian population, whilst other markers under investigation in IL-1α, IL-

1β and IL-1RN were not associated with either CP or AgP.(Karasneh et al. 2011)

In the above study the authors could not confirm the role of IL-1β in the periodontal pathologies in the Jordanian people, but it was the only one to fail this relation.

Interleukin-6 (IL6) is proven to be a powerful stimulator of osteoclast differentiation and bone resorption in the oral cavity.

Production of IL6 is modulated by polymorphisms, and higher levels of this cytokine are found locally in patients with chronic periodontitis. In the study of Farhat, a complete physical mapping of the IL6 gene was made, to identify the polymorphisms associated with chronic periodontitis. The result suggested that allele G of polymorphism rs2069837 (located in the second intron of IL6) was a suggestive marker of protection against chronic periodontitis in a Brazilian population sample (Farhat et al. 2017b) supporting the findings of Scapoli in the Italian population.

In the European population Hodge had also studied the IL parameters with different results from Scapoli et al. The aim of his research was to examine IL1α and IL-1β genetic polymorphisms in unrelated European white Caucasian patients with generalized early-onset periodontitis (GEOP). He concluded that there was a lack of any association between the IL1 polymorphisms and GEOP in the population presented, calling into doubt the usefulness of these candidate genes as markers of susceptibility to this form of periodontitis. (Hodge, Riggio, and Kinane 2001)

Interleukin 6 (IL6) is indeed a major mediator of the host response to tissue injury, infection and bone resorption in the oral cavity. In a study by Kurtis, gingival crevicular fluid (GCF) level of IL6 were determined in patients with non-insulin dependent diabetes mellitus (NIDDM) with periodontitis, adult periodontitis, and healthy controls by means of an enzyme linked immunosorbent assay (ELISA) similar to our study. No correlation was found between GCF IL6 levels and all clinical parameters. These findings suggested that GCF IL6 levels were significantly higher in the area of inflammation and in local periodontal destruction. (B Kurtiş et al. 1999)

The study of the IL role in periodontal pathogenesis, soon passed from the prevention state to the therapeutic phase. If in the pathological states, the literature showed us that the levels of IL-1β and IL6 were higher, the first studies to report the impact of mechanical cleaning came to similar conclusions, corroborating the theory behind the pivotal role of IL.

To study the impact on periodontal therapy on IL levels, a study was made to quantitatively assess the effect of initial periodontal therapy on gingival crevicular fluid levels. Clinical examinations were performed and gingival crevicular fluid samples obtained from six subjects with generalized severe chronic periodontitis prior to initial periodontal therapy and at re-evaluation (6-8 weeks).

Gingival crevicular fluid interleukin (IL)-1α and IL-1β were the only cytokines to differ in initially diseased vs. initially healthy sites. The results confirm that periodontal therapy effectively reduces pro-inflammatory cytokines and chemokines, including less well-described mediators that may be important in the initiation and progression of periodontitis. (Thunell et al. 2010)

Another study of periodontal therapy showed that after periodontal therapy, IL-1β levels were significantly reduced in the moderate and deep pocket sites having concluded that periodontal treatment improves the clinical parameters, and this improvement is evident in deep pocket sites. These results confirmed that IL-1β is effective for evaluating periodontal inflammation and can thus be used as a laboratory tool for assessing the activity of periodontal disease. (Toker, Marakoglu, and Poyraz 2006)

Fentoglu evaluated the effects of periodontal treatment on serum and gingival crevicular fluid (GCF), pro-inflammatory cytokine levels in hyperlipidemic patients with periodontitis. A significant decrease was also found in GCF IL6 at the end of the study period in the HS group, the conclusion being that periodontal therapy and antilipidemic treatment may bring about beneficial effects on the metabolic and inflammatory control of hyperlipidemia. (Fentoğlu et al. 2012)

One of the therapies for some pathologic states of periodontal disease is the use of AINES (anti-inflammatory drugs). The aim of the Toker study was to

determine the effects of meloxicam after initial periodontal treatment on interleukin-1β (IL-1β) and IL-1 receptor antagonist (IL-1ra) in gingival crevicular fluid (GCF) and concluded that there were no significant differences between the two groups in any of the investigated parameters. Their observations showed there was no evidence of meloxicam influencing levels of IL-1β and IL-1α in chronic periodontitis. (Toker, Marakoglu, and Poyraz 2006)

1.18.9. Interleukins in Other Oral Cavity Pathologies

The crucial role of IL in the oral cavity goes well beyond the periodontal tissues. Cytokines are associated with levels of inflammation and not only affect periodontal/peri-implant sites but also involve other types of oral pathology such as caries, pulpal, and periapical tissue destruction.

In a study by Dill, in a sample of 136 cases with deep carious lesions and periapical lesions (cases) and 180 Nine single-nucleotide polymorphisms in IL-1β, IL6, TNF, RANK, RANKL, and genes were selected for genotyping variations. The study concluded that IL-1β may be associated with periapical lesion formation in individuals with untreated deep carious lesions. (Dill et al. 2015)

Another study to prove the role of IL in oral pathology, consisted of 41 patients including 22 volunteers who were currently smokers and 19 volunteer non-smokers.

The Ebru study demonstrated that cigarette smoking increases the amount of dental plaque over time in smokers and does not influence GCF contents of IL6 and TNF-alpha. (Ebru Olgun Erdemir, Duran, and Haliloglu 2004)

Whole salivary interleukin (IL)-1β and IL6 in smokers and those who have never smoked with early stage diabetes is also a field of Interleukin investigation.

The idea in this thesis was to assess periodontal status and whole salivary IL-1β and IL6 level and the results show that among controls, periodontal inflammation was worse, and whole salivary IL-1β and IL6 levels are higher in smokers than those who have never-smoked. Among patients with prediabetes, periodontal inflammation and whole salivary IL-1β and IL6 levels were

comparable between smokers and non-smokers. (Javed et al. 2015)

In special needs patients with severe forms of oral cavity infections, a study was made to assess periodontal disease status of individuals with Downs syndrome (DS). The authors concluded that carriage of the IL-1 rare alleles in the Downs subjects tended to confer a protective effect against loss of periodontal attachment. (Khocht et al. 2011).

1.18.10. Inflammatory Differences in Peri-implant tissues and Periodontal Tissues

The role of interleukin ot may be pivotal to the long-term survival rate of implant-based restorations, not only in periodontal tissues but also in peri-implant tissues

As in the PCF, the PICF was also studied in relation to inflammatory mediators and cytokine levels.

A number of authors have studied the role of inflammation and auto-immune disease through interleukins measurements. In one of the most cited articles, peri-implant gingival healing following one-stage implant placement was investigated and compared to periodontal healing in a sample of forty patients. Inflammatory markers of periodontal surgical sites increased at week one, decreasing significantly during early healing and continually decreased in late healing (weeks 6-12). IL6, IL-8, MIP-1β and TIMP-1 levels significantly increased at surgical sites at week one, significantly decreasing thereafter. Week one IL6, IL-8 and MIP-1β levels were three times higher at implant sites, with the conclusion that the differences observed suggested, that peri-implant tissues, compared to periodontal tissues, represent a higher pro-inflammatory state. (Emecen-Huja et al. 2013)

Another pilot study also tried to map the differences of peri-implant sites compared to periodontal sites in terms of interleukin presence. The study was conducted to determine levels of inflammatory cytokines in crevicular fluid from healthy implants and those implants affected by peri-implantitis from a sample of fifty implants from 13 patients. Interleukin-1β was detected in the crevicular

fluid of implants in all three groups (healthy = 59,47 ± 15,55 pg/site; early peri-implantitis = 460,77 ± 35,67 pg/site; and advanced peri-implantitis = 191,10 ± 21,60 pg/site) indicating that IL-1β is present in implant gingival crevicular fluid and may be modulating attachment loss in implants suffering from peri-implantitis (Panagakos et al. 2017).

1.18.11. Inflammatory PICF Levels in a Healthy State and in Active Disease (perimplantitis and mucositis)

Since Panagakos, a number of publications have studied PICF in healthy and pathological states.

Ata-Ali analyzed the clinical, microbiological, and immunological aspects in the peri-implant sulcus fluid PICF of patients with healthy dental implants and patients with peri-implantitis. Samples were obtained from 24 peri-implantitis sites and 54 healthy peri-implant sites in this prospective cross-sectional study. PICF samples were analyzed for the quantification of Interleukin (IL)-8, IL-1β, IL6, IL-10 and Tumor Necrosis Factor (TNF- α) using flow cytometry IL-1β, IL6, IL-10 and TNF-α and were significantly higher at the sites with peri-implantitis compared to healthy peri-implant tissue, suggesting that the peri-implant immune response could contribute to bone loss in peri-implantitis.

A significant relationship was observed between the concentration of cytokines (interleukins 1β, 6 and 10 and TNF-α) and the inflammatory response in peri-implantitis tissue. (Ata-Ali et al. 2015)

Several authors have also tried to find a correlation between the presence of interleukins and the presence of peri-implant disease, but with no concrete results. In one of those studies the investigators evaluated interleukin-1β (IL-1 β) and interleukin-6 (IL6) concentration in crevicular fluid, and the impact of gene polymorphisms on healthy and diseased implants in comparison to healthy teeth. The author examined 47 implants and teeth and found no significant difference in the concentration of IL-1β and IL6 detected between groups. Moreover there was no correlation between the concentration of IL-1β and IL6 in the crevicular sulcular fluid present in healthy or diseased

osseointegrated implants in comparison with healthy teeth. (Melo, Lopes, Shibli, Marcantonio, et al. 2012).

The impact of inflammatory levels on the success of dental implants was also presented by Vaz, again with no concrete result. The study aimed to analyze the association between polymorphisms in the IL1 gene cluster and failure of dental implants in a Portuguese population sample. The prevalence of the polymorphisms -889 IL-1α gene and +3953 IL-1β gene, determined by the positive result of TGP (Genetic Test for Periodontitis; CGC, Genetics, Portugal), in the sample rehabilitated with dental implants was 33.50%. Allele 1 of the IL-1β gene was the most prevalent (62.20%), followed by allele 1 of the IL1α gene (54.80%) and the least frequent was allele 2 of IL-1β gene (37.40%). The authors concluded that the alleles 1 and 2 of IL1α gene and the alleles 1 and 2 of IL-1β gene were statistically associated with the success or lack thereof, of the dental implants. (Vaz et al. 2012)

Another research paper found no correlation between implant failure and IL-1β presence in a retrospective observational, prevalence study in 58 edentulous Caucasian patients rehabilitated with implant overdentures. A total of 229 implants were included in the study and the prediction model included the following variables: mean probing depth, metal exposure, IL-1β allele2, maxillary edentulous, and Fusobacterium nucleatum. The F. nucleatum was the only factor that showed significant links to the outcome while the inflammatory molecules registered none. (Sampaio Fernandes et al. 2017)

A similar study by Dirschnabel et al. showed another borderline impact of cytokine and implant loss in a sample composed of 277 - 92 subjects with implant loss, and a control group - 185 subjects with no implant loss. There was no difference between the groups with or without implant loss taking into account genotype alleles for IL-1β (C-511T) polymorphism. When individuals reporting up to a single implant failure (n=254) were investigated compared to patients presenting multiple implant loss (n=23), no difference was observed between groups for genotype and allele frequencies. This suggests further IL1 haplotype analysis is needed to clarify the global involvement of IL-1 proteins in the osseointegration modulation process. (Dirschnabel et al. 2011)

On a higher level of evidence, the results tend towards the opposite direction. A systematic review of interleukins and most common cytokines released in crevicular fluid was conducted from 1996 up to and including October 2013 (with the literature completed in 2015 on the healthy and peri-implant affected sites).The aim of this study was to answer two clinical questions: 1) whether patients with peri-implantitis (PP) present higher prevalence of any specific inflammatory cytokine in peri-implant crevicular fluid (PICF) when compared to healthy patients; and 2) whether local inflammation measured in PICF can be used as a predictor for disease.

Different interleukins respond differently to pathology states. For example, (IL)-1β and IL-10 expressions are in inverse states in peri-implant crevicular fluid (PICF) in healthy and diseased regions. The inflammatory process around implants and the influence of this process on clinical diagnosis reported lower IL-1β and higher IL-10 levels characterized by healthy peri-implant conditions. In so doing it highlighted the predominance of anti-inflammatory processes in sites with no signs of disease. IL-10 levels decreased significantly according to the increase in disease status. These levels can therefore help to differentiate between healthy tissue, mucositis, and periimplantitis, indicating that interleukins may be useful as a biochemical marker for early diagnosis of peri-implant disease. (Casado et al. 2013b)

Another study defending this conclusion examined the PICF levels of interleukin-1β (IL-1β), tumor necrosis factor alpha (TNF-alpha), interleukin-8 (IL-8) and macrophage inflammatory protein-1alpha (MIP-1alpha) in patients with non-manifesting inflammation, early and late stages of mucositis. A positive correlation was noted in the control group between IL-1β and TNF-alpha and between MIP-1alpha and IL-8 in the group with early stage mucositis. The results suggest that cytokines could be prognostic markers of implant failure. (Petković et al. 2010)

On a higher level of evidence in a split-mouth study investigating the relationship between the concentration of IL-1β in gingival crevicular fluid (GCF) and peri-implant crevicular fluid (PICF), findings indicated that the level of IL-1β may be an important supplement to clinical findings in measuring the health status of gingival or peri-implant tissues. (Yaghobee, Khorsand, and Paknejad

2013)

It is thus difficult to find a clear correlation between interleukins and the state of health or the presence of peri-implant disease, although the trend seems to indicate that interleukins exist in higher concentrations in heightened disease conditions.

The aim of the Hultin study was to characterize microbiota and inflammatory host response around implants and teeth in patients with peri-implantitis. They included 17 partially edentulous patients with a total of 98 implants, of which 45 reported marginal bone loss of more than three fixture threads after the first year of loading. Nineteen subjects with stable marginal tissue conditions served as controls. The concentrations of IL-1 beta were approximately the same at the different sites. (Hultin et al. 2002)

The results of this systematic review revealed that Interleukin (IL)-1β was the most observed cytokine (n = 12), followed by tumor necrosis factor (TNF)-α (n = 10). Other cytokines were also linked to peri-implantitis, such as IL-4, IL6, IL-8, IL-10, IL-12, and IL-17. Statistical differences were revealed when IL-1β release was compared between healthy implant sites and peri-implant disease or mucositis sites. When perimplantitis and mucositis were compared, no statistical differences could be detected. The conclusion reached was that crevicular fluid containing inflammatory mediators, such as IL-1β and TNF-α, can be used as additional criteria for a more robust diagnosis of peri-implant infection. In addition, once the inflammatory process was in place, no differences were found between peri-implant mucositis and perimplantitis (Faot et al. 2015).

1.18.12. The inflammatory reaction arising from the fitting the abutment several times

The problem of connecting and disconnecting the final abutment and the impact on marginal bone loss was put forward by a Swedish research group under Lindhe (I Abrahamsson et al. 2002). Biochemical (interleukin/inflammatory mediators) level was also investigated with the aim of the study being to

examine the effects of abutment change on inflammatory cytokine production around implants.

IL-1β level and probing depths were lower in test group patients compared to the control group patients, with the conclusion that the delivery of the final abutment at the second surgery would induce less inflammation in the tissues around the implant. (Kuppusamy et al. 2015)

1.18.13. The inflammatory reaction of Placing the implant at different crestal levels (subcrestal, equicrestal and supracrestal)

The position of the implant in relation to bone position (supra, infra or equicrestal) was also measured from the perspective of the interleukins in a total of 27 dental implants placed subcrestaly in 21 periodontally healthy patients. Repeated clinical and cytokine measurements were obtained over 12 months. The levels of interleukin (IL)-4, -6, -10, and -12p70, tumor necrosis factor-α, and interferon-γ in gingival crevicular fluid and peri-implant crevicular fluid were not significantly different and did not vary over time. The conclusion was that following subcrestal implant placement, the immune response of peri-implant and periodontal tissues is similar when cytokine is assessed. (Nogueira-Filho et al. 2014)

1.18.14. The effect of Oral Hygiene on the Inflammatory levels

The lack of oral hygiene and the impact on interleukins concentration was seen in 25 patients where samples of gingival crevicular fluid and peri-implant fluid were collected in the sulcus of the tooth and of the implant after undergoing professional hygiene treatment. After the no-hygiene phase (21 days) a second sample of GCF and PICF was collected and after 69 days of the resumption of oral hygiene techniques, a third sample was taken. The study concluded that the volume of the crevicular fluids increased significantly after 21 days of plaque accumulation around teeth and implants and decreased significantly by 69 days. A significant increase of IL-1β was observed after plaque accumulation

around the teeth, whereas in the implant site the increase was not statistically significant. These data suggest that increased volumes of gingival and periodontal fluid could be useful markers of early inflammation in gingival and peri-implant tissues. In the presence of de novo plaque, implants showed lower, and almost significant levels of IL-1β compared with natural teeth. (Schierano et al. 2008)

A number of publications began to analyze the impact of inflammation in daily implant procedures through interleukins measurement. One publication aimed to calculate the amounts of neutrophil elastase and interleukin-1β (IL-1β) in the crevicular fluid of dental implants that were placed and restored immediately after extraction of teeth. The absolute values remained unchanged during the early and late follow-up periods. Paired analysis showed that the absolute values in the periodontal crevicular fluid were similar when compared to the corresponding samples obtained during the early and the late follow-up periods. These findings suggest that, despite being an invasive procedure, the placement of implants according to the immediate loading protocol, does not provoke an inflammatory reaction. (Gruber, Nadir, and Haas 2010)

Another study looked for levels of inflammatory enzymes in gingival crevicular fluid between natural teeth and endosseous dental implants and between well-integrated and failing implants. The results of this study indicated that neutrophil elastase, myeloperoxidase, and beta-glucuronidase levels in gingival crevicular fluid appear to be good candidates for study as risk markers of implant failure. (Boutros et al. 2017)

1.18.15. Interleukin influence on Different abutment material

Inflammatory reaction can be theoretically modeled in terms of constitution of biomaterial.

Dental implant abutments are fundamental prosthetic components in dentistry that require optimal biocompatibility. The primary aim of the Baracwz cross-sectional study was to undertake a preliminary assessment of differences in the pro-inflammatory cytokine and bone metabolism mediator protein expression in

the peri-implant crevicular fluid (PICF) adjacent to transmucosal abutments. Multivariable analyses reported no evidence of a group (titanium or zirconia), gender, or age effect with regard to the expression of pro-inflammatory mediators under evaluation. Significant differences were observed for the bone mediator leptin, with titanium abutments demonstrating significantly elevated levels when compared to zirconia. Osteopontin showed a significant correlation to the age of the subjects. The conclusion was that no significant differences in pro-inflammatory cytokine or bone metabolism mediator profiles were observed biochemically, with the exception of leptin, for the titanium and zirconia abutment materials. (Barwacz et al. 2015)

The molecular PICF findings support the clinical biocompatibility of both titanium and zirconia abutments observed leading the way for other studies.

A clinical and immunohistochemically study designed by Dellavia characterized the cellular and molecular patterns for bone and soft tissue loss surrounding implants restored with different implant platform configurations. These used abutments with the following mismatches: 0 mm (control group), 0.25 mm (test group (1), 0.5 mm (test group (2) and 0.85 mm (test group (3).

The author concluded that following prolonged exposure of abutments in the oral cavity, the configuration of the implant abutment interface does not seem to affect the inflammatory cellular and molecular pattern responsible for bone loss. (Dellavia et al. 2013)

It is interesting that inflammation may have an influence on soft tissue healing around implants. The objective of the Linkevicius review in 2015 was to analyze research regarding the effect of zirconia and titanium as abutment material on soft peri-implant tissues. The outcome measures were (1) soft tissue color, (2) soft tissue recession, (3) peri-implant probing, (4) bleeding on probing, (5) esthetic indexes, (6) patient-reported outcome, (7) marginal bone level, and (8) biological complications. The author concluded that the research does not support any obvious advantage for Ti or Zr abutments over each other. However, there is a significant tendency in Zr abutments to bring about a better color response in the peri-implant mucosa and superior esthetic outcome measured in the PES score. (Linkevicius and Vaitelis 2015)

In 2017 the same author undertook a systematic review with the aim of evaluating available evidence for a difference in the stability of peri-implant tissues between titanium abutments in relation to gold alloy, zirconium oxide, or aluminum oxide abutments. This broader study revealed that titanium abutments did not give rise to a higher bone level in comparison to gold alloy, aluminum oxide, or zirconium oxide abutments. The authors stated however, that there was a lack of information on the clinical performance of zirconium oxide and gold alloy abutments as compared to titanium abutments. (Linkevicius and Apse 2017)

On the soft tissue level, the aim of the human study by Degidi, was to conduct a comparative immunohistochemically evaluation of vascular endothelial growth factor (VEGF) and nitric oxide synthase (NOS) expression, inflammatory infiltrate, proliferative activity expression, and microvessel density (MVD) in peri-implant soft tissues of titanium and zirconium oxide healing caps. In their specimens, the inflammatory infiltrate was mostly present in the titanium specimens. The data indicate that the higher expression of these two mediators could be correlated to the higher amount of bacteria present around the titanium samples. (Degidi et al. 2006)

Bieleman studied the inflammatory response map in narrow implants placed in the mandible anterior region of 30 edentulous patients. Samples from the PICF were collected 1, 2, 4, 8, and 12 weeks after surgery and analyzed for IL-1β, IL6, IL-10, and TNF-α levels using ELISAs. IL-1β concentrations showed a short-lived peak after the first week, particularly in atrophic patients and sites with bone type I. The IL6 concentrations peaked in the 1st and 2nd weeks in atrophic patients and in bone type II. (Bielemann et al. 2017)

1.18.16. Interleukin influence on Oral pathology

Interleukins are not only important in periodontal or peri-implant biology. In oral pathology there is also a vast field of research in cytokines behavior.

In 2008 SahebJamee compared the concentration of tumor necrosis factor alpha, interleukin 1α, 6, and 8 in the saliva of oral squamous cell carcinoma

patients.

The concentration of salivary interleukin 6 in oral squamous cell carcinoma patients was higher than the control group than the experimental group as was the concentration of salivary tumor necrosis factor alpha, interleukin 1α and 8. These results show that more studies are needed to accept the usefulness of these cytokines in the prediction and diagnosis of oral squamous cell carcinoma or evaluation of treatment. (SahebJamee et al. 2008)

Researchers have also attempted to establish the relationship between levels of salivary and serum interleukin (IL6) in autoimmune diseases such as lichen planus. These authors conducted a systematic review and meta-analysis to compare levels of saliva and serum IL6 among patients with OLP and healthy control participants. The results of the meta-analysis revealed significant differences in the levels of IL6 in saliva and serum between patients with OLP and healthy control participants, asserting that levels of IL6 in saliva and serum may be potential biomarkers for OLP. However, additional research is needed to confirm findings of this meta-analysis. (Liu et al. 2017)

This research objective is also found in a study of pre-malignant lesions (PML) and oral carcinoma in a non-interventional case control study carried out with the aim of exploring saliva as a diagnostic medium for detecting interleukins (IL) 6 and 8 as biomarkers. A significant co-relation was found for qualitative salivary detection of IL6 and IL-8 among the groups. In terms of quantitative salivary concentrations of leukotrienes, no significant co-relation was found in levels of IL6 among the groups while there was significant association in IL-8 levels between the groups. They concluded that salivary detection of IL6 & IL-8 could be used as probable biomarker for early detection of oral PML & OSCC in an etiologically distinct Pakistani population. (Khyani et al. 2017)

This tendency is also found in bone metastasis of prostatic and kidney tumors and in epithelial-mesenchymal transition (EMT), a biological process associated with cancer stem-like or cancer-initiating cell formation which contributes to the invasiveness, metastasis, drug resistance, and recurrence of malignant tumors. Jiang reported elevated interleukin (IL6) signals that were differentially expressed in the stromal compartment of the follicular ameloblastoma. These findings suggest that IL6 promotes tumor-stem like cell formation by inducing,

implying a role in the etiology and progression of the benign but locally invasive neoplasm. (Jiang et al. 2017)

Meghji studied three human cell lines derived from oro-pharyngeal squamous cell carcinomas of the head in oral cavity tumors such as ameloblastoma and soft tissue tumors for bone-resorbing activity in vitro. These results indicate that IL1 is responsible for the prostaglandin-independent bone resorbing activity synthesized by these cells in vitro, and may contribute to the bone destruction associated with the tumor. (Meghji et al. 1988)

1.18.17. Interleukin influence on Partial removable dentures

Aslo reported interesting associations between soft tissue response around natural teeth supporting dentures in investigating the periodontal status and susceptibility to periodontal disease progression of the teeth in contact with removable partial dentures (RPD) in a comparison with control teeth in unrestored mouths with a partial denture. By means of both clinical parameters and interleukin IL-1β levels in gingival crevicular the authors concluded RPDs are a risk factor for periodontal disease progression because of increased plaque accumulation associated with increased total IL-1β levels and impaired clinical periodontal parameters. (Bülent Kurtiş et al. 2017)

Another study from the same series evaluated the gingival crevicular fluid (GCF) contents of interleukin-6 (IL6) and interleukin-8 (IL-8) and the clinical parameters of the teeth supporting fixed partial dentures (FPD) and the contralateral teeth in order to assess the effect of scaling and root planning (SRP) on clinical parameters and the GCF levels of cytokines. The non-surgical periodontal treatment reduced the total amount of IL-8, but not IL6, and the clinical parameters of the teeth with FPD and contralateral teeth. Therefore, a regular program for dental prophylaxis is also important for the maintenance of periodontal health in patients with FPD. (E O Erdemir et al. 2010)

CHAPTER 2. OVERALL OBJECTIVE OF THE RESEARCH

The overall objective of this thesis was to study auto-immune response, after an alloplastic material (a titanium dental implant) is placed into bone, and evaluate early healing osseointegration protocols on the soft tissue level.

We divided the thesis into two parts: Part 1 (the animal study) where the objective was to calibrate cytokines extraction protocol methodology and set a baseline of IL-1β and IL6 concentrations present in periodontal fluid, in peri-implant crevicular fluid and in blood samples. It also served to calibrate ELISA cytokine reading. The accuracy of the reading protocols was checked, and a baseline of interleukin concentrations was set. The sample transportation was check marked in dry ice and was feasible and then calibrated to a sample handling protocol.

In this section the feasibility of setting a sample size calculation for the statistical significance of the RCT was checked. (this was difficult due to the nature of sheep healing as opposed to the human healing response)

In part 2 of the protocol a clinical randomized trial was designed to compare the behaviour of IL-1β and IL6 inflammatory/autoimmune impact on different biomaterials (Z, A or T) in contact with the connective tissue of the peri-implant biological width. These results were set against two control groups, the IL6 and IL-1β inflammation on the periodontal healthy sulcus and the IL-1β and IL6 blood concentrations at the time of implant placement.

The goal of these measurements of inflammation in the human study was to research the direct impact on marginal bone resorption.

As secondary outcomes in the RCT, biological width, age, gender, anatomical position (maxilla vs mandible), duration of surgery and primary/secondary stability were compared.

The main objective was to be able to formulate a clinical recommendation that will help clinicians to provide a proper evidence-based approach method and see if it makes any difference the type of biomaterial choice over dental implants.

CHAPTER 3. RESEARCH PROJECT – PART 1 - ANIMAL STUDY

SECTION 3.1 OBJECTIVES AND HYPOTHESIS OF THE ANIMAL EXPERIMENTAL MODEL

3.1.1. Animal Study (AS) Objectives

To evaluate Changes in inflammatory fluid levels of Interleukin 1 beta (IL-1β) and Interleukin 6 (IL6) from T0 (baseline) to T1 (1month) to T3 (3 months) according to the following PICO question:

(P) In Sheep receiving an abutment over a dental implant, does **(I)** CAD-CAM zirconia when compared to CAD-CAM titanium and CAD-CAM acrylic provide equal inflammation during **(O)** the osseointegration period Animal Study **(S)**?

3.1.2. AS Hypothesis

Primary Outcome Measures: Relate the influence of abutment material on the peri-implant inflammation in accordance with the following assumptions:

3.1.3. AS Specific aim 1: Study Inflammatory reaction at Implant Placement (T0 Baseline)

H0: There is no difference in the production of an inflammatory reaction of IL6 and IL-1β at T0, of CAD-CAM titanium when compared to CAD-CAM zirconia or CAD-CAM acrylic healing abutments placed over dental implants under the standard protocol.

H1: There is a difference in the production of an inflammatory reaction of IL6 and IL-1β at T0, of CAD-CAM titanium when compared to CAD-CAM zirconia or CAD-CAM acrylic healing abutments placed over dental implants under the standard protocol.

3.1.4. AS Specific aim 2: Study Inflammatory reaction at T1 (1Month)

H0: There is no difference in the production of an inflammatory reaction of IL6 and IL-1β in T1, of CAD-CAM titanium when compared to CAD-CAM zirconia or CAD-CAM acrylic healing abutments placed over dental implants under the standard protocol.

H1: There is a difference in the production of an inflammatory reaction of IL6 and IL-1β in T1, of CAD-CAM titanium when compared to CAD-CAM zirconia or CAD-CAM acrylic healing abutments placed over dental implants under the standard protocol.

3.1.5. AS Specific aim 3: Study Inflammatory reaction at T3 (3 Month)

H0: There is no difference in the production of an inflammatory reaction of IL6 and IL-1β at T3, of CAD-CAM titanium when compared to CAD-CAM zirconia or CAD-CAM acrylic healing abutments placed over dental implants under the standard protocol.

H1: There is a difference in the production of an inflammatory reaction of IL6 and IL-1β at T3, of CAD-CAM titanium when compared to CAD-CAM zirconia or CAD-CAM acrylic healing abutments placed over dental implants under the standard protocol.

3.1.6. AS Specific aim 4: Study Inflammatory reaction from T0 to T3

H0: There is no difference in the production of an inflammatory reaction of IL6 and IL-1β from T0 to T3, of CAD-CAM titanium when compared to CAD-CAM zirconia or CAD-CAM acrylic healing abutments placed over dental implants under the standard protocol.

H1: There is a difference in the production of an inflammatory reaction of IL6 and IL-1β from T0 to T3, of CAD-CAM titanium when compared to CAD-CAM zirconia or CAD-CAM acrylic healing abutments placed over dental implants under the standard protocol.

3.1.7. AS Study Experimental Design

The experimental outline took place in three major phases. The first was a preparatory phase that involved protocol clearance from the Scientific Council of the Lisbon School of Dentistry, University of Lisbon, INIAV Instituto Nacional de Investigação Agrária e Veterinária, and ORBEA, the animal ethical committee.

The second phase involved the experimental animal work at the Estação Zootécnica de Santarém. This phase had three key elements: implant placement at baseline, one-month sample extraction and 3-month final sample extraction.

The third phase took place at the Instituto Superior Técnico (IST-School of Engenering, Lisbon Portugal) for interleukin sample reading.

The study follows the guidelines shown in table 1.

Table 1 - Study Summary - Autoimmune host response Animal Study

Permission INIAV Permission ORBEA Scientific Council FMDUL Permission		
Group Formation CAD-CAM Zirconia Healing Abutments n=12	Group Formation CAD-CAM Titanium Healing Abutments n=12	Group Formation CAD-CAM Acrylic Healing Abutments n=12
Implant and abutment Placement (T0) At estação zootécnica nacional Santarém		
Characterization, Inflammatory levels		
Baseline (T0)	1 month (T1)	3 month (T3)
Characterization, Inflammatory levels	Characterization, Inflammatory levels	Characterization, Inflammatory levels
Cytokines Reading Instituto Superior Técnico (IST) Bioengineering and Biochemistry Department		

3.1.8. AS Research Unit Location - Estação Zootécnica Nacional Santarém

FIGURE 1 - Estação Zootécnica Nacional (EZN) in Santarém (Portugal), where the study took place under an agreement established with the University of Lisbon College of Dentistry (FMDUL)

This experimental animal study was carried out at the Estação Zootécnica Nacional (EZN) in Santarém (Portugal), under an agreement established with the University of Lisbon College of Dentistry (FMDUL). A license was requested from Direção Geral de Veterinária and was accepted (DGV) on the 21/04/2016 DGV/D8GA; 0421/000/000/2016 (Appendix A).

This station has a long tradition and in-depth knowledge of experimental animal designs and has provided a solid foundation for countless PhD theses undertaken at the University of Lisbon College of Dentistry.

3.1.9. AS and Human Study (HS) base center - Lisbon University, School of dentistry (FMDUL)

FIGURE 2 - University of Lisbon School of Dentistry in Lisbon

The center of investigation for the animal study and for the Clinical trial was conducted in Lisbon University School of Dentistry, within the Oral Surgery and Implantology post-graduation program. The Specialization Course in Implantology and Oral surgery trains health professionals, enabling them to fully address the oral rehabilitation needs of the general population. The program of this course follows rules outlined by the majority of associations of specialties within the field of Oral Surgery and Implantology both European and North American and complies with the Community Directive that regulates the profession of Dentists and their specialties (78/686/EEC directive council of July 25, 1978), particularly in the requirement of 3 years full time study. Students in this course will be among clinicians who participate in the study.

The College of Dental Medicine at the University of Lisbon was the first college dedicated to graduate dentists in Portugal. It currently qualifies Dental Hygienists and Prosthetic technicians. For Dental Medicine a Master and Doctorate programs are available. At the College of Dental Medicine researchers and clinicians work together to bring the most recent science and technology to bear in their practice. The recently remodeling of the Biomedical and Oral Sciences Research Unit and the Clinical facilities optimizes this output, encouraging scientific research within the courses specializations and contributing to the protection of public health and welfare of the population in the field of oral health. Several protocols with institutions, agencies and public or private services and other individuals are being carried out to achieve this goal.

Research Unit: Oral and Biomedical Sciences Research Unit, University of Lisbon College of Dentistry (FMDUL). Implant placement and the fluid extraction for the clinical trial were all completed at the Oral surgery and implantology unit.

3.1.10. AS and HS sample readings - Instituto Superior Técnico - University of Engineering (IST)

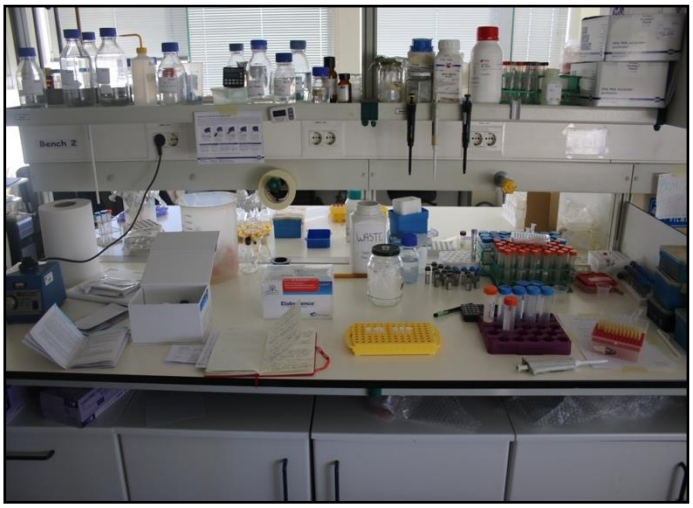


FIGURE 3 - Instituto Superior Técnico Laboratory of Biochemical Investigation in Lisbon

FIGURE 4 - Instituto Superior Técnico Laboratory of Biochemical Investigation in Lisbon (outside view)

Our team worked with the Institute for Bioengineering and Biosciences (IBB) pole, a research unit at Instituto Superior Técnico (IST), Universidade de Lisboa (UL), with an international reputation for cutting edge research and strategic advanced education in fundamental and applied biological sciences, biotechnology and bioengineering, exploring innovative approaches to key scientific and technological questions in biosciences and bioengineering and transforming scientific knowledge into tangible innovation.

The Institute was created in 2013, through the integration of the Bioengineering Research Group (BERG) and the Biological Sciences Research Group (BSRG), two research groups established at IST in 1991.

We directly worked with Prof. Gabriel Monteiro´s team in the biochemistry department on the 6th Floor of the Bioengineering pole.

As part of the team we had a P_{hD} Student in genetics and biochemistry Dr. Sofia Duarte to help us follow the protocols and verify the results.

All the samples were stored and prepared in the department. Storage for the biological samples was done in the -80ºC freezer while the ELISA reagents were in the -20-freezer chamber.

All cytokine readings of the animal study and RCT were done on site with very strong cooperation and strict protocol control.

SECTION 3.2 MATERIALS AND METHODS

3.2.1. Animal experimental model

Six Sheep were used in this study. These animals were approximately 3 years of age and had a body weight of 10-12 kg.

FIGURE 5 - 6 Sheep were used for this study. The image on the left shows the box where sheep were housed during the study. The image on the right shows the weighing boxes before surgery was being performed.

All animals were free from disease and followed the international requirements for animal well-being.

Throughout the experimental study, all animals were kept on a soft diet and subject to oral hygiene protocols by means of mechanical cleaning of both teeth and implants. The choice of sheep as the animal model was based in successful studies on osseointegration using this animal model. (Stocchero et al. 2017)(Yoo et al. 2014)(Galli et al. 2015)

The sheep were part of an experiment with dental implants placed in the mandible and although the baseline reading was equal to all, the subsequent readings at T1 and T3, were done in a serial reading protocol.

As an initial baseline (T0) all implants were placed in sheep and subjected to cytokine extraction methodology.

At T1, 2 sheep were anesthetized and subjected to cytokine extraction methodology.

At T3, 4 sheep were anesthetized and were subjected to cytokine extraction methodology.

Animal sheep experiments were approved by the Animal Ethical Committee and fulfill all legal requirements.

Ethical Clearance was obtained from ORBEA and Protocol Clearance from INIAV Direcção Geral de Alimentação e Veterinária/Instituto nacional de investigação agrária e veterinária was obtained.

The protocol was also approved by the Scientific Commission of the Faculdade de Medicina Dentária de Lisboa (FMDUL)

The sheep model followed a strict protocol of implant placement (Biomet-Zimmer® T3 Implant) and Abutment placement (Zirconia, Acrylic and Titanium).

Inflammation harvest protocol included sterilized periopaper® in the peri-implant sulcus on the day of surgery, 60 minutes after the last stitch was tied (T0), at 1 Month (T1) and at 3 Month (T3). The harvested inflammation fluids were transported in dry ice to IST (Instituto Superior Técnico) where they were frozen to -80ºC until sample reading.

3.2.2. Implant and Abutment In Vitro Mechanical Characterization

3.2.2.1. Experimental Dental Implant Characterization

Three implants (Biomet-Zimmer ® Platform Switch T3) were placed on both sides of the sheep mandible and a two-piece healing abutment of different biomaterial (Z, A or T) was randomly screw-retained on top of those implants.

Implant microgeometry presented a textured surface. They were sandblasted with calcium phosphate particles by means of calcium phosphate spheres size 75 µ (large grit) and bathed in a nitric acid (acid etch) solution.

The sample size was 12 implants in each experimental and control group, three on each hemi-mandible, making a total of 36 implants.

The abutments were randomly assigned prior to surgery for all 6 sheep. The 36 implants and abutments were inserted in random order, from sheep 1 to sheep 6, left side to right side, from distal to mesial. One zirconia, titanium and acrylic in each hemi-mandible.

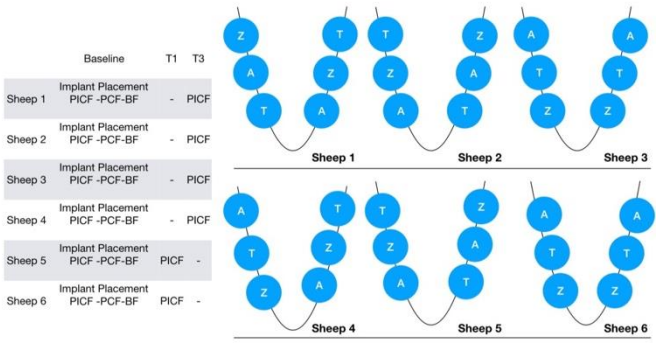


FIGURE 6 - Abutment placement schedule prior to surgery. Table represents procedure roster for each sheep.

3.2.2.2. Implant Scanning Electron Microscopy (SEM) characterization

In order to control quality and be sure of implant microgeometry, the implants were scanned at SEM. 5 implants from the same lot were randomly chosen for SEM analysis, similar to the ones that were going to be used in the study.

As shown in fig. 7, the implant has different rough patterns over its surface. The microgeometry of the surface technology has some varied features along the height of the implant, the collar has a rough morphology (1 - 3 microns) from dual acid-etching (DAE) and the body is composed of a rougher part (10 microns) via resorbable calcium phosphate media blast.

The images 8 to 11 show the outer craters made by the resorbable spheres on the implant body and the pattern of the rough surface created by the acid bath.

It has an internal platform-switching with an outer diameter of 4.1mm and an inner diameter of 3,25 mm. It is a single thread design on a conical type morphology. (fig.12).

The experimental implant was used both in the animal study and in the RCT.

At SEM, there were no alterations in microgeometry on the implant samples.

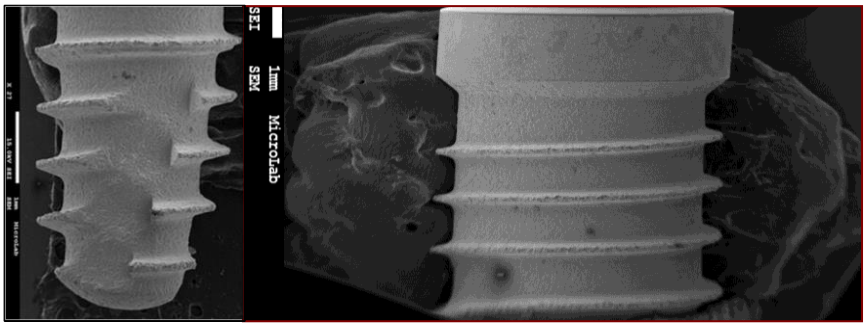


FIGURE 7 - SEM analysis on the implant surface 27X times magnification. Notice the sandblasted and Acid Etched surface of the T3 Biomet-Zimmer® Implant. A conical type of implant with an internal connection with platform-switch.

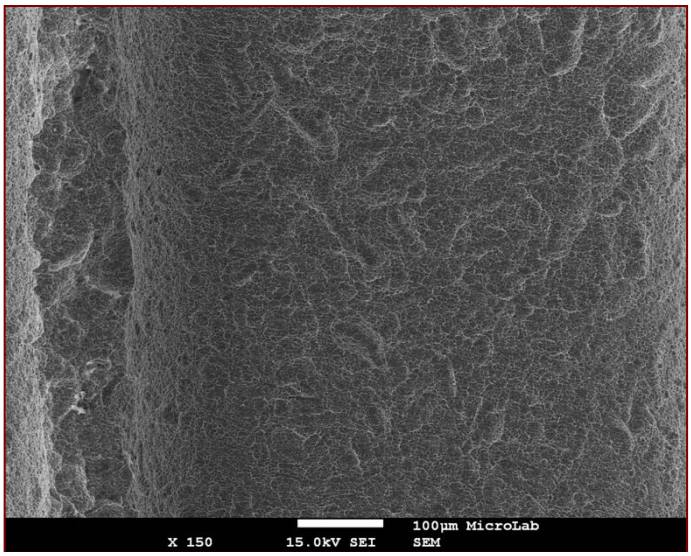


FIGURE 8 - Biomet-Zimmer® T3 surface at 150x Magnification. We can see the microgeometry produce by sandblasting and acid-etching the surface.

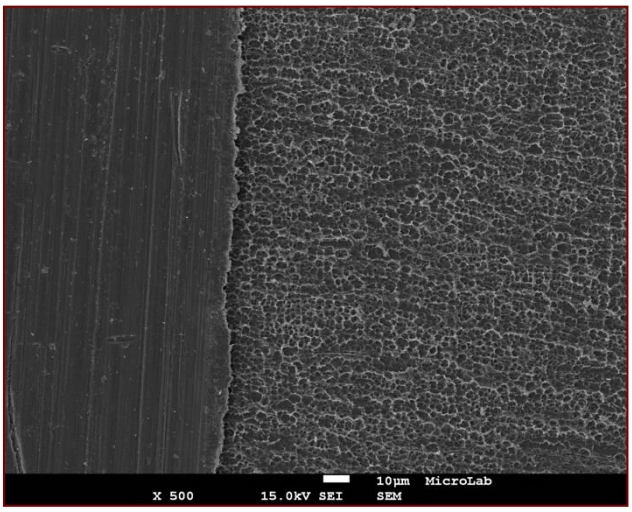


FIGURE 9 - Transition of the smooth to rough surface at x500 Magnification. Close-up of the transition of the acid-etch surface to the machined collar of the Biomet-Zimmer® T3 Implant**.**

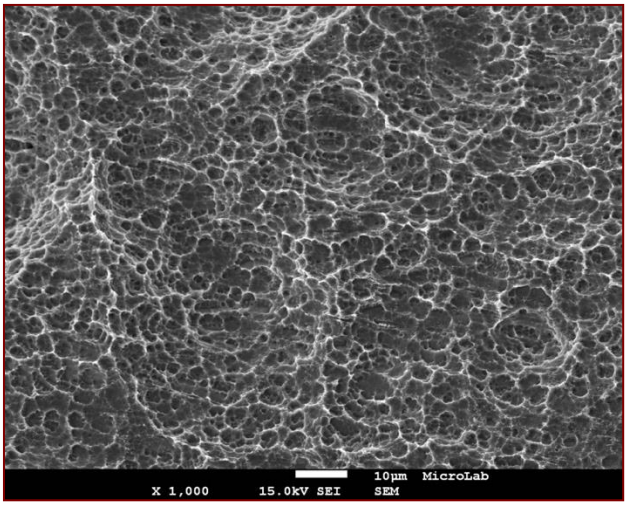


FIGURE 10 - Sandblasted, Acid etched surface at 1,000 magnifications. Surface topography of the Biomet-Zimmer® T3 Implant.

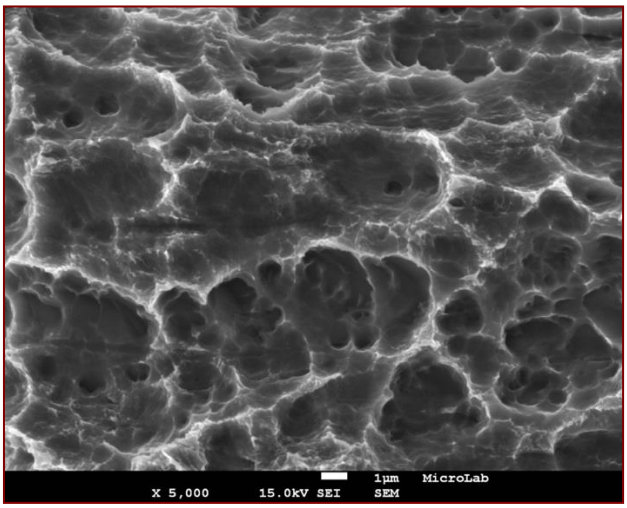


FIGURE 11 - Biomet Zimmer ®T3 implant at x 5,000 Magnification**.**

3.2.2.3. CAD-CAM Healing Abutment SEM - Scanning Electron Microscopy Characterization and fabrication

SEM was undertaken to exercise close control over the healing abutments which were all similar in construction ensuring that the CAD-CAM duplication protocol didn´t alter the micro and macrogeometry.

Microscopic and radiological characterization for abutment evaluation was performed, and both microgap and macrogeometry accuracy were checked.

Two healing abutments were selected from each material, making a total of 6 abutments scanned for microscopy.

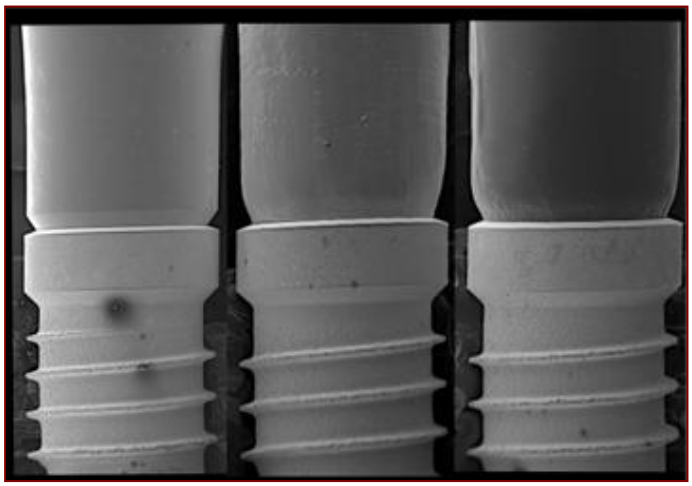


FIGURE 12 - SEM microscopy of the three-different implant abutment connections. From left to right: titanium, acrylic and zirconia. The complex abutment-implant was also used in the RCT study.

Passivity could be checked under the SEM at 27, 150 and 500 times magnification on the three types of healing abutments

To confirm passive fit, each healing abutment that came out of the milling machine (either titanium, acrylic or zirconia) was checked for quality. They were all inserted in an analog, tightened with a hand-torque and viewed under light magnification lupes. Those that did not meet the criteria were discarded and another was created.

FIGURE 13 - Placement of a CAD-CAM titanium healing abutment on an analog for passivity check.

3.2.2.4. CAD-CAM Titanium Healing Abutment

The titanium abutment was obtained from the Biomet-Zimmer® implant company.

It is a two-piece Encode® abutment that serves as a healing abutment. (fig.14)

The platform width dimensions were 3,25 mm (matching the 3,25 mm implant platform, since platform-switching implants were used, the implant diameter was 4,1 mm, despite the connection being 3,25 mm) with a height of 4 mm as shown in fig. 15.

Figure 15 and 16 show the different magnifications and platform geometry design for the microgap characterization in titanium

This two-piece healing abutment, pre-fabricated by the implant company (Biomet-Zimmer®) has a passivity of less than 1 micron at an angle of 35° with the major axis of the implant and an angle of 75° between the platform and the surface of the implant.

FIGURE 14 - Two-piece titanium healing abutment. Biomet-Zimmer® two-piece Encode ® abutment.

Figure 15, 16 and 17 show the different magnifications and platform geometry design for the microgap characterization on titanium.

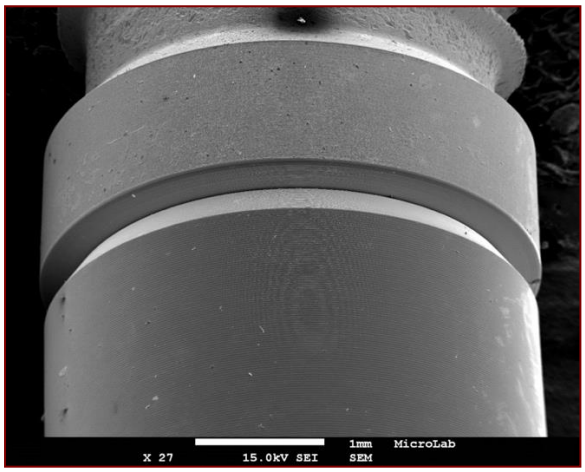


FIGURE 15 - Implant abutment connection with a titanium healing abutment at 27x magnification.

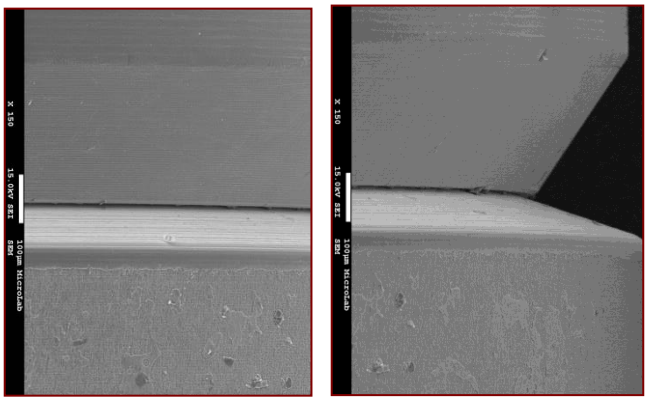


FIGURE 16 - Implant abutment connection with a titanium healing abutment at 150x magnification. Note the almost perfect fit of the implant to the abutment surface.

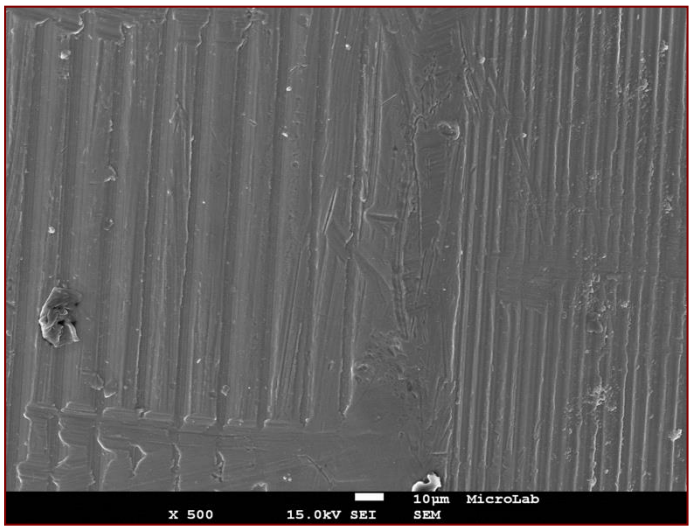


FIGURE 17 - Implant abutment surface of a titanium healing abutment at 500x magnification. Note the marks made by the bur.

3.2.2.5. CAD-CAM Zirconia Healing Abutment

A two-piece CAD-CAM titanium Encode® abutment was read by the optical scanner of a milling machine (Zirkonzhan™) (fig. 18,19), a block of zirconium oxide (zirkonzhan ™) was milled to create an identical zirconia healing abutment. (fig. 20,21)

The passivity of the abutment was confirmed and was very similar to the titanium, with an angle of 35° to the major axis of the implant and an angle of 75º with the implant platform. (fig. 22 to 25)

FIGURE 18 - Optical digital reading CAD-CAM production of Zirconia and Acrylic two-piece healing abutments

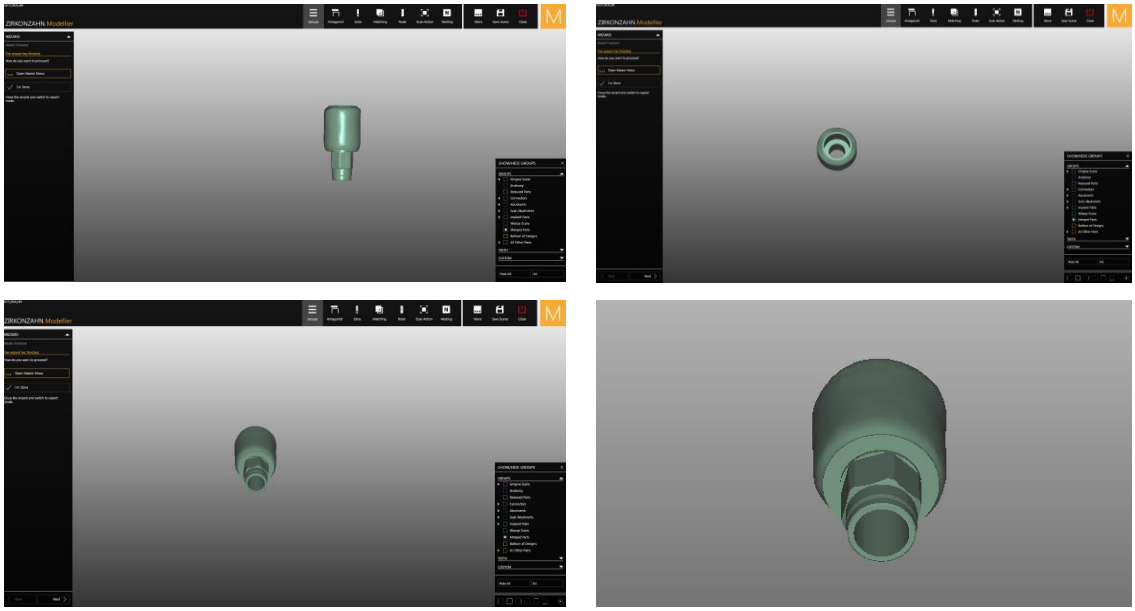


FIGURE 19 - Software CAD-CAM production of Zirconia and Acrylic two-piece healing abutments

FIGURE 20 - CAD-CAM Zirconia Disc before abutment process

FIGURE 21 - Clinical two-piece CAD-CAM Zirconia healing abutment

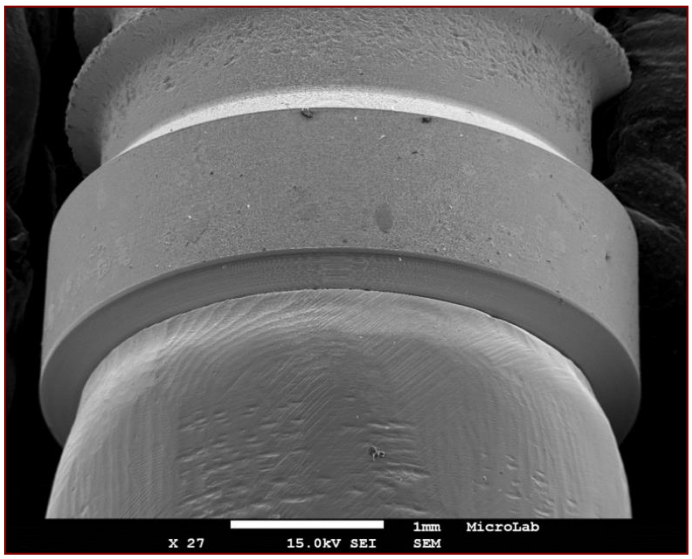


FIGURE 22 - Implant abutment connection with a CAD-CAM zirconia healing abutment at 27x magnification. Note that the fit is very similar to CAD-CAM Titanium

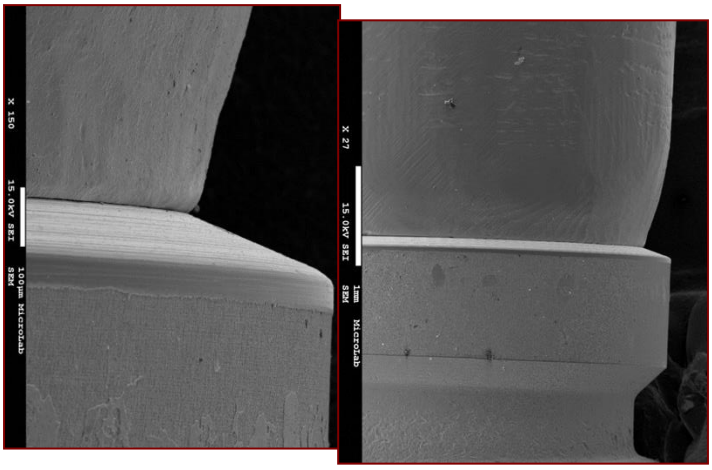


FIGURE 23 - Implant abutment connection with a CAD-CAM zirconia healing abutment at 150x magnification. Note the almost perfect fit of the Zirconia to titanium Surface

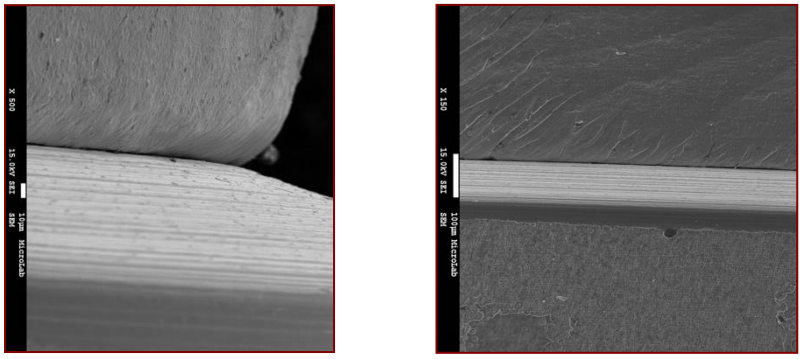


FIGURE 24 - Microgap present at magnification of 500x when using a CAD-CAM zirconia healing abutment

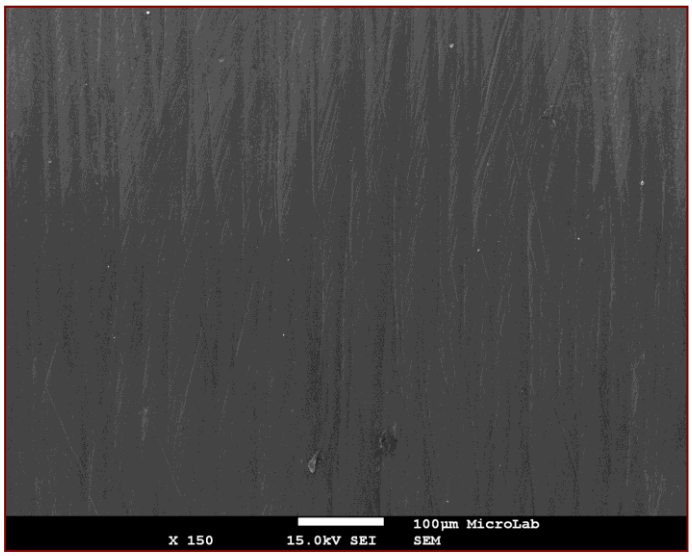


FIGURE 25 - Implant abutment surface of a CAD-CAM Zirconia healing abutment at 500x magnification. Note that the marks made by milling machine burs are smoother that the titanium ones.

3.2.2.6. CAD-CAM Acrylic Healing Abutment

A two-piece titanium Encode ® abutment was read by the optical scanner of a milling machine (zirkonzhan™) and a block of polymethyl "temp-premium" (zirkonzhan™) (fig. 27) was milled identical to the titanium abutment. This showed the worst passivity compared to T and Z (a larger "microgap"), with an angle of 41º to the axis of the implant, and an angle of 87 º to the implant platform. However, it still performed well, compared to cast abutments. (fig. 28-31)

FIGURE 26 -Clinical CAD-CAM Acrylic healing abutment

FIGURE 27 - CAD-CAM Acrylic Disc before abutment process

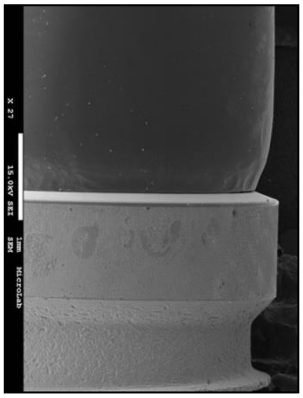


FIGURE 28 - Implant abutment connection with an CAD-CAM Acrylic healing abutment at 150x magnification. Note the almost perfect fit of the CAD-CAM Acrylic to titanium surface of the implant platform

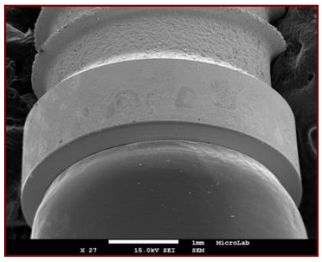


FIGURE 29 - Implant abutment connection with a CAD-CAM Acrylic healing abutment at 27x magnification

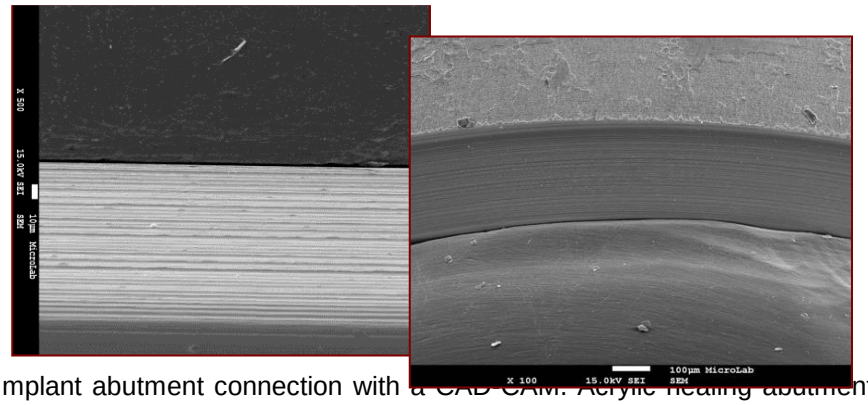


FIGURE 30 - Implant abutment connection with a CAD-CAM Acrylic healing abutment at 27x magnification

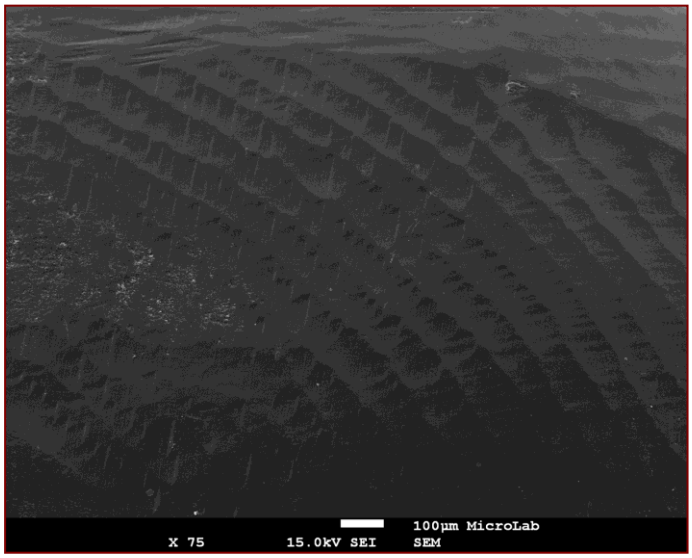


FIGURE 31 - Implant abutment surface of a CAD-CAM Acrylic healing abutment at 500x magnification. Note the marks made by milling machine burs that seem to “take “small pieces out unlike the carving pattern on the CAD-CAM zirconia abutment

3.2.3. Sheep Anatomical Study Pre-Surgical procedure

At the Estação Zootécnica Nacional (EZN) in Santarém (Portugal) the team had the opportunity to source the skull of a sheep with approximately the same age of the sheep in our study and with the aid of tomography cone-beam technology was able to use it for implant site planning and preparation.

The diastema of the sheep mandible is a thick bicortical plate with a central area corresponding to the inferior alveolar nerve that culminatess at the mental foramen (fig. 32).

On the sagittal view, the buccal lingual dimensions average 10 mm from cortical to cortical. The most anterior area held one impacted canine in each hemi-arch while the middle area and posterior space was limited by the inferior alveolar canal. (fig. 33 to 35)

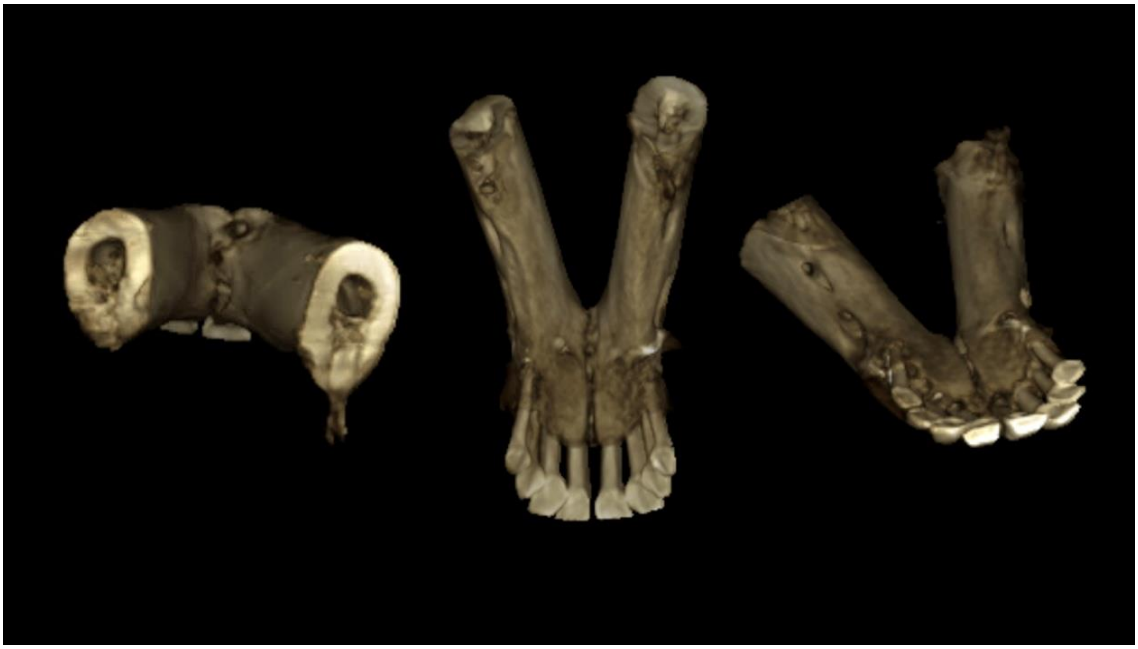


FIGURE 32 - CBCT scan of sheep model 3D reconstruction

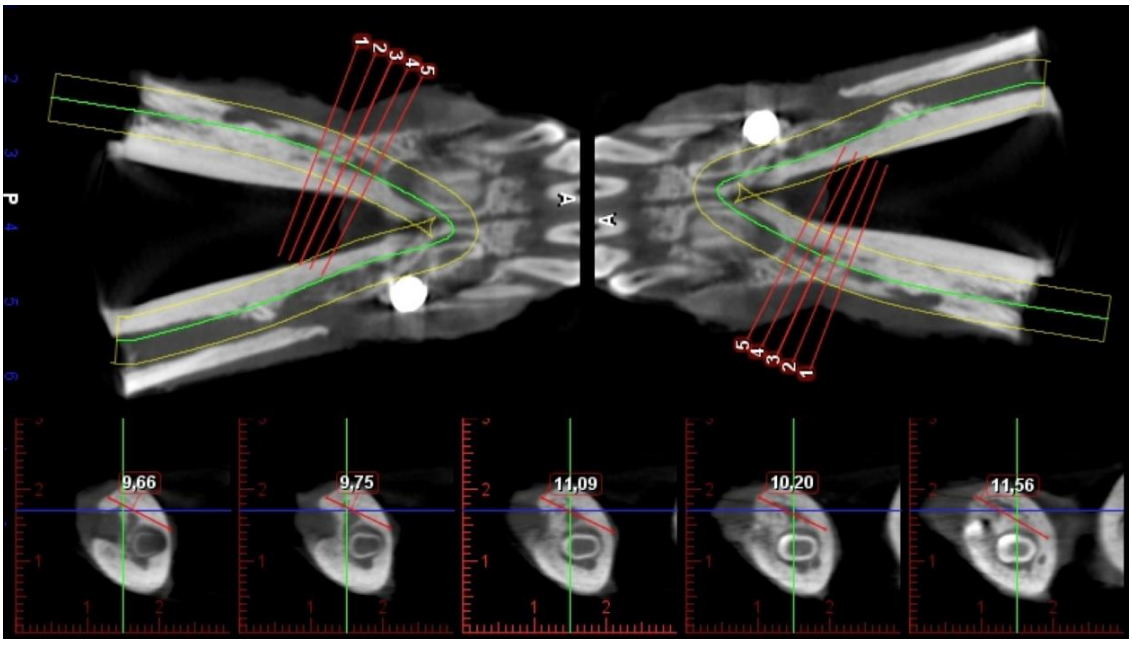


FIGURE 33 - Anterior (zone Diastema) for implant placement. Note the anterior zone near the incisor area had some impacted teeth and a very prominent mental foramen and inferior alveolar nerve canal.

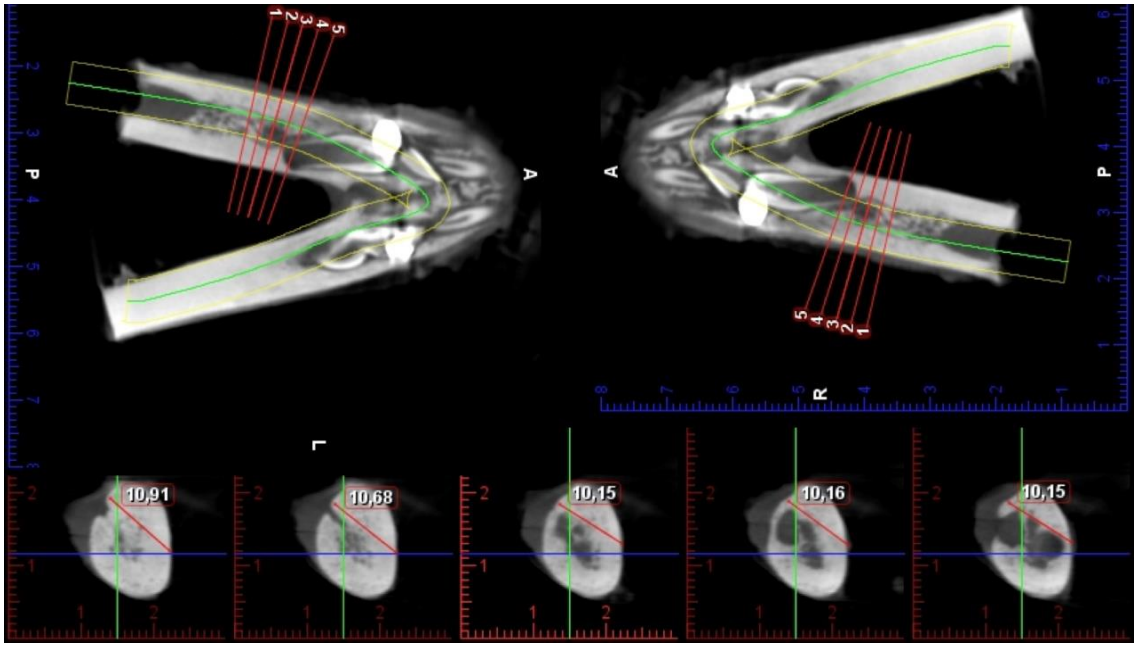


FIGURE 34 - The middle area of the diastema for the middle implant. The area presented much more trabecular and cortical bone than the anterior area near the mental foramen with an average of 10,5 mm from buccal to the lingual cortex.

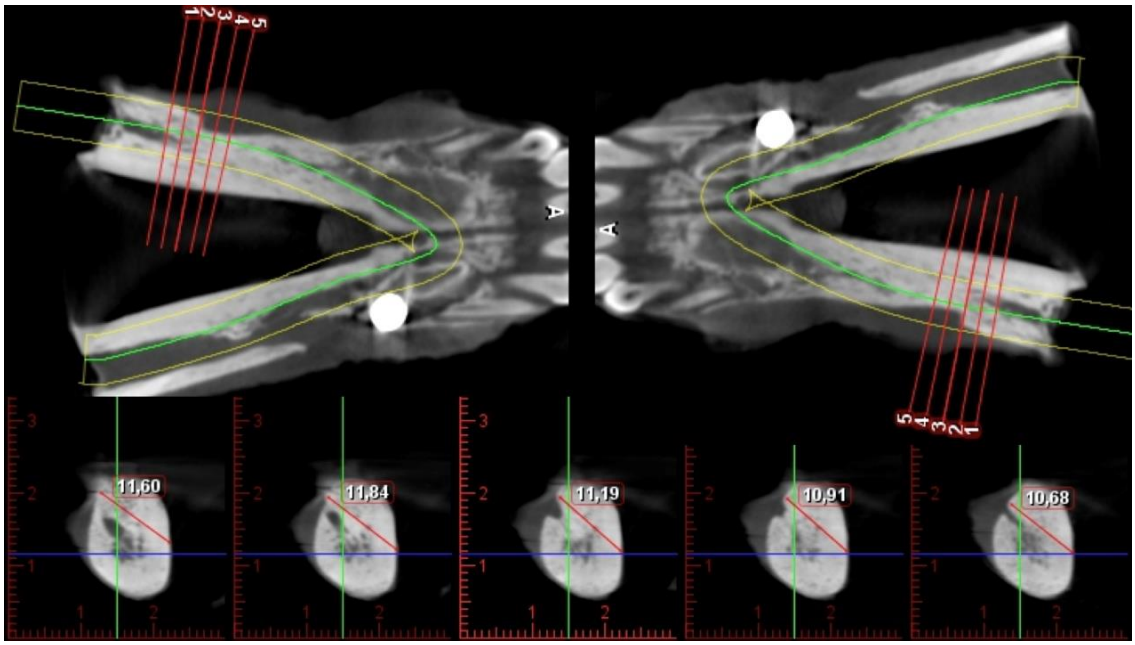


FIGURE 35 - Posterior area for implant placement. The posterior area is the best bone for implant placement since the inferior alveolar canal is narrower in this area and the influence of molar teeth enlarge the area of the buccal to lingual dimensions. Note that there is almost 12 mm width in some areas.

3.2.4. Surgical procedure Description

General Anesthesia: All surgical procedures (implant placement and sample collection) were performed under general anesthesia. The sheep were placed in the operating theatre, on a heated pad, with general anesthesia via intravenous Pentothal.

The Anesthetic protocol for all Sheep, initiated with Acepromazine 5 mg/ml (Calmivete VÉTOQUINO–France) for tranquilization, Thiopental Sodium (Pentothal-Braun-Germany) for induction and Isoflurane (Abbott-Laboratórios Lda - Alfragide) administered for maintenance.

A cuffed endotracheal tube was used for intubation, a gas mixture of isoflurane was supplied and the process monitored with an electrocardiogram.

Following general anesthetic, the sheep was anesthetized locally via arthicaine cloridrate 4% and epinephrine 1:100.00 (Laboratórios Inibsa, Barcelona/Espanha) administered subperiosteally in the buccal and lingual of the sheep mandible (left and right). (fig. 36)

The latency time was 130 seconds before starting any surgical procedure.

FIGURE 36 - Sheep gross anatomy. Endotracheal tube and subperiosteal local anesthesia

Implant placement: Surgical preparation procedure was initiated with bone crest sounding (with an endodontic file nº40), measured with a periodontal probe (North Carolina probe, Hu-Friedy, Germany). (fig. 37 and 38)

Crestal incision with a 15c blade was made in the diastema (anatomical part of the sheep mandible), between the premolars and incisors. (fig. 39)

The incision was made on the buccinator muscle, 4 mm below the transitional area from the lingual papillae, corresponding to the upper 1/3 of the lateral wall of the mandible. (fig. 37)

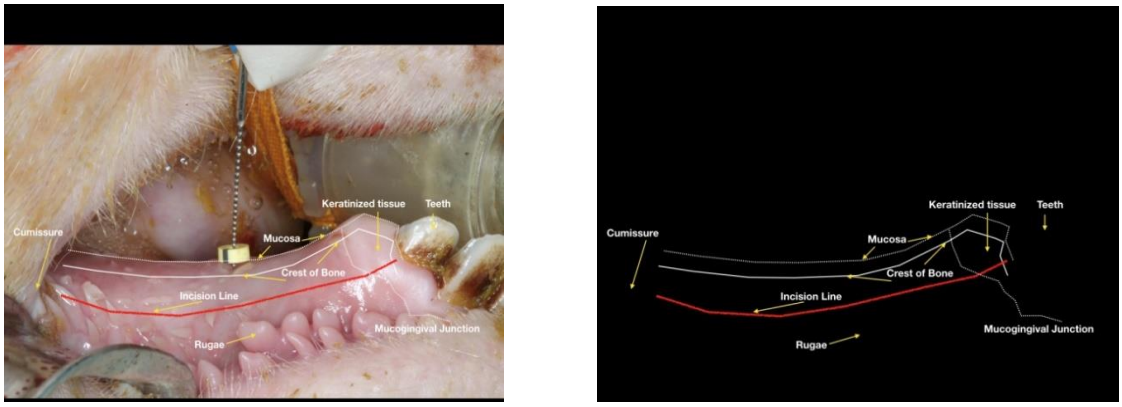


FIGURE 37 - Sheep gross anatomy. Red Line marks the incision line for the correct implant position - Left to right: clinical view and Diagram.

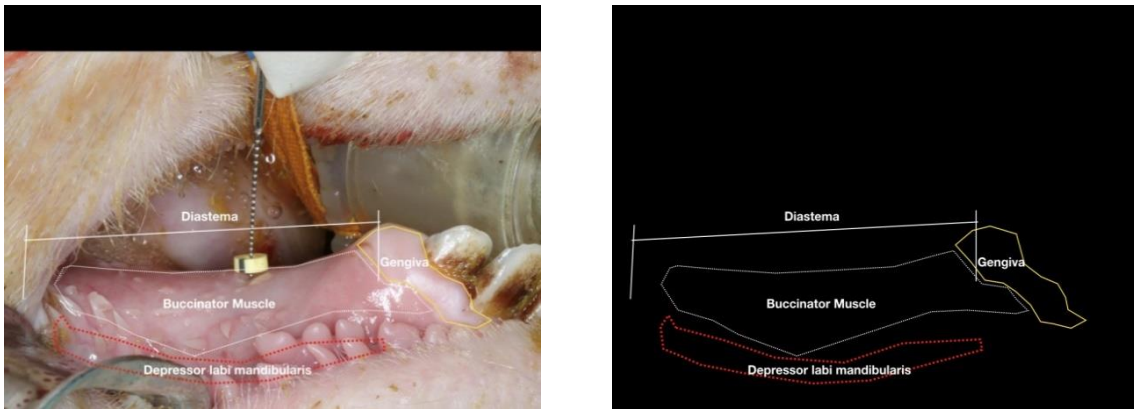


FIGURE 38 - Sheep gross anatomy. Muscle Position and Diastema Characterization Left to right: clinical view and Diagram.

The incision was made from above the depressor labbi mandibularis and below the zygomaticus muscle. Mesial-Distal the incision was made anterior to the masseter and posterior to the commissural insertion of the orbicularis oris muscle. (fig. 38)

A full-thickness mucoperiosteal flap with periosteum retractors for basal bone access was created and the mental foramen was isolated.

FIGURE 39 - Full-Thickness Mucoperiosteal flap with a 15c blade for basal bone access.

The flap was retracted to the lingual border of the mandible to gain access to the medial wall.

The osteotomies were drilled according to the implant manufacturers specifications (Biomet-Zimmer® T3 Implant with platform-switch).

Firstly, a round bur marked the initial drilling area by making a small indentation on the basal bone. Secondly, a 2-mm cylindrical initial bur was used to achieve full bony depth. The thirdly, step of the conical/tapered flute burs, the 3,25-special bur for T3 tapered implants was used and finally, the 4.1 bur was used.

Implant insertion was done with a low-speed device, and torque never exceeded 50 n/cm^2. (fig. 40 and 41)

FIGURE 40 - Animal study surgical tray at Baseline (T0). Notice the implant motor and surgical kit as well the prosthodontic torque system. The Osstell® unit for primary stability measurements is shown at lower right.

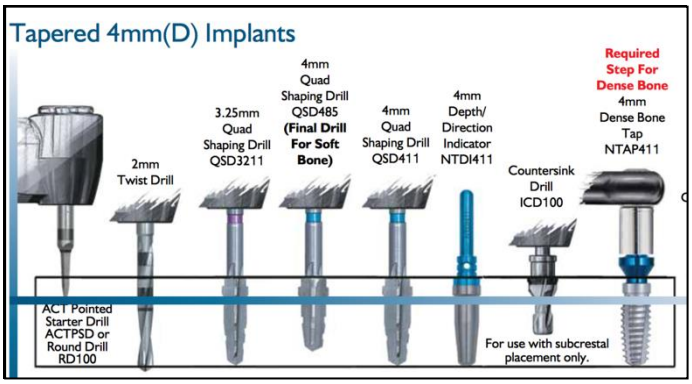


FIGURE 41 - Implant T3 Biomet-Zimmer® Drilling protocol according to the manufacturer (courtesy of Biomet-Zimmer)

Implants were placed in an approximately equidistant linear position in relation to neighboring implants using a pre-calibrated caliper. The two implants that were placed distal to the mental foramen of the sheep mandible were 10 mm apart from each other, while the one in front of the mental foramen was 30 mm from the one in the middle. (fig. 42 and 43)

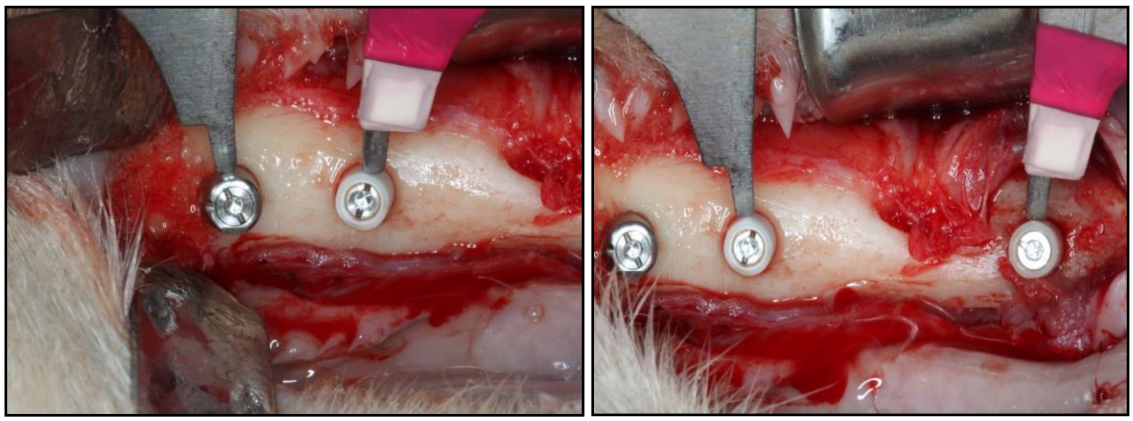


FIGURE 42 - Equidistant linear implant positions. The most posterior positioned implant is placed 10 mm from the adjacent tooth, the middle is placed 10 mm from the most posterior one and the most anterior placed 30 mm from the middle.

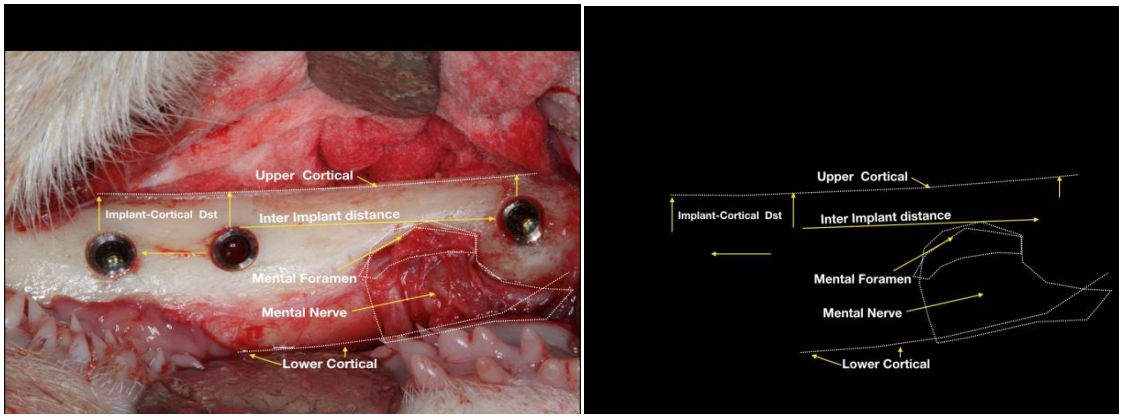


FIGURE 43 - Sheep gross anatomy. Open flap retraction, implant position in relation to bonelandmarks and mental nerve exposure. Left to right: clinical view and Diagram

Care was taken not to overheat the bone by using a low drilling speed of approximately 800 rpm with external sodium chloride irrigation.

Implants were placed 1 mm below the marginal crest and received the respective healing abutments (1 Zirconia,1 Acrylic and 1 Titanium) (fig. 44). The implants were placed 1 mm below the crestal bone and not 2 mm as in the RCT due to sheep cortical bone being very thin. In order to engage bone with higher primary stability it was critical to place them in this position for maximum primary stability.

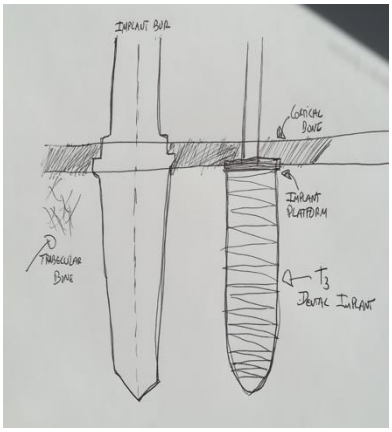


FIGURE 44 - Subcrestal position in Biomet-Zimmer T3 Implants (adapted from Biomet-Zimmer®).

The objective was for the titanium part of the implant to stay in bone and only the abutment material to be in contact with the soft tissues.

The area was sutured with Vicryl 4,0 (interrupted sutures) for primary wound closure.

After suture was completed a chlorhexidine gel was used to wipe the area clean. (fig. 45 to 48)

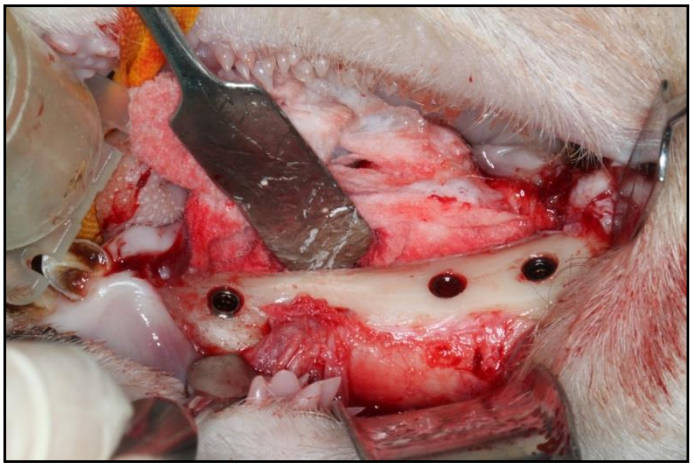


FIGURE 45 - All three implants were placed in the Sheep Mandible according to plan. Notice the exuberant emergence of the mental nerve through the mental foramen.

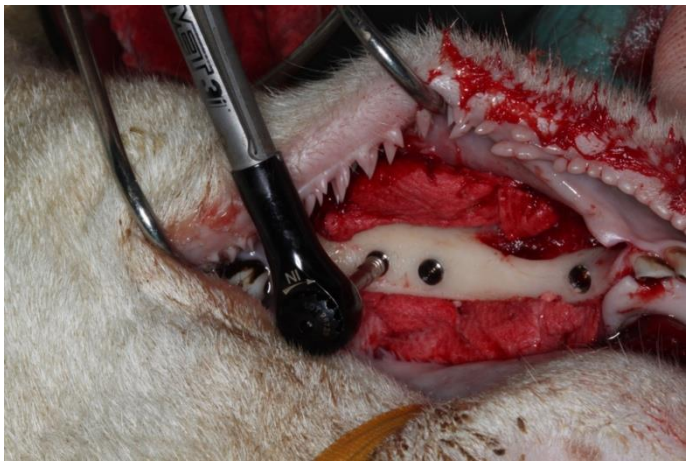


FIGURE 45A - All three implants were placed in the Sheep Mandible according to plan. Torque control

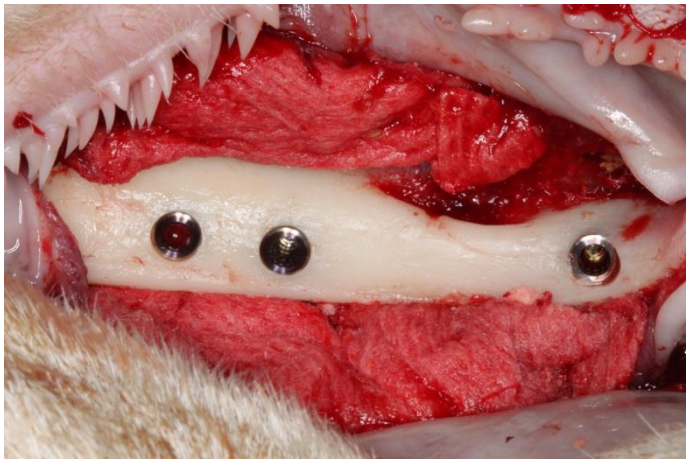


FIGURE 45B - All three implants were placed in the Sheep Mandible according to plan. Lateral View

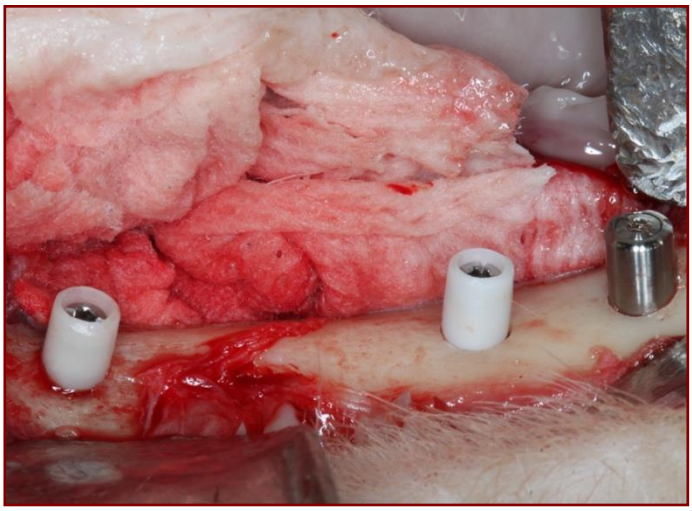


FIGURE 46 - Implant positions with randomly placed CAD-CAM Zirconia, CAD-CAM Acrylic and CAD-CAM Titanium abutments.

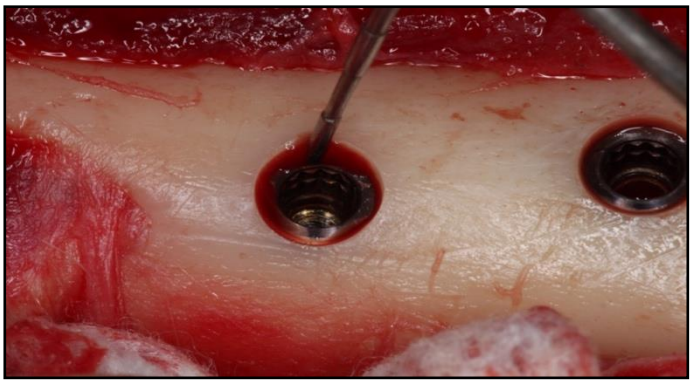
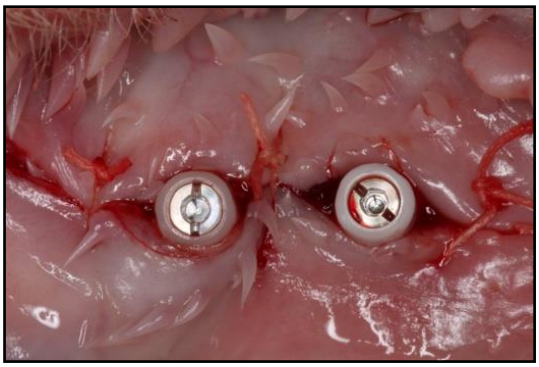


FIGURE 47 - Close-up of the subcrestally placed implants, 1mm below marginal bone.



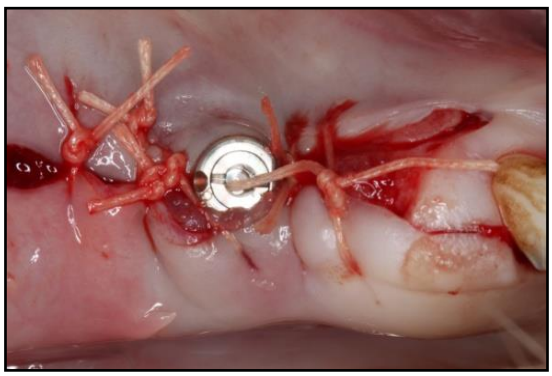


FIGURE 48 - Primary wound healing control with resorbable suture

3.2.5. Experimental animal diet, oral hygiene protocol and post-op.

Postoperative instructions: After implant placement, animals were given postoperative therapy that included antibiotic administration with long lasting oxytetracycline (Oxymycin LA 300 mg/ml Norbrook Laboratories). The dosage protocol included two treatments every 3 days. For analgesia – Caprofen 100 mg (Rimadyl-Pfizer) was administered in two treatments over two consecutive days.

During the healing period, surgical wounds were checked frequently in order to search for signs of complications or infections. A soft diet was used during the healing period. A plaque control program was initiated and maintained throughout the study: every week a check-up and professional cleaning was performed.

All the post-op controls were undertaken by the principal investigator and by a veterinarian from the Santarém Research institute.

3.2.6. PICF and PCF Cytokines Extraction Method at T0

Five types of inflammatory sample (for IL-1β and IL6) readings were done:

1-Perimplant tissue fluid for CAD-CAM Zirconia Abutments (fig. 54)

2-Perimplant tissue fluid for CAD-CAM Acrylic Abutments (fig. 52)

3-Perimplant tissue fluid for CAD-CAM Titanium Abutments (fig. 53)

4-Periodontal Inflammatory fluid from teeth (Control) (fig. 51)

5-Blood Samples (Control)

At T0, the extraction methodology for the peri-implant abutment fluid involved a waiting period of 60 min after the final stich was completed. Following this, 4 stripes of periopaper were placed directly in the mesial, distal, buccal and lingual peri implant sites.

PCF extraction was also undertaken at this stage for T0 periodontal cytokine characterization. The protocol involved isolation with cotton rolls, placing a

periopaper® strip in the periodontal sulcus for 20 seconds to discharge the initial exudate.

After 20 seconds, a Periopaper ® tip 1 mm was inserted inside the sulcus until a slight resistance was felt for 20 seconds. Four strips (4) were placed in an Eppendorf tube for T0 periodontal interleukin baseline reading.

Immediately following incision, blood fluid samples were drawn, with four periopaper strips in the center of the incision.

The protocol was undertaken for all abutments placed as shown in fig. 50 to 54.

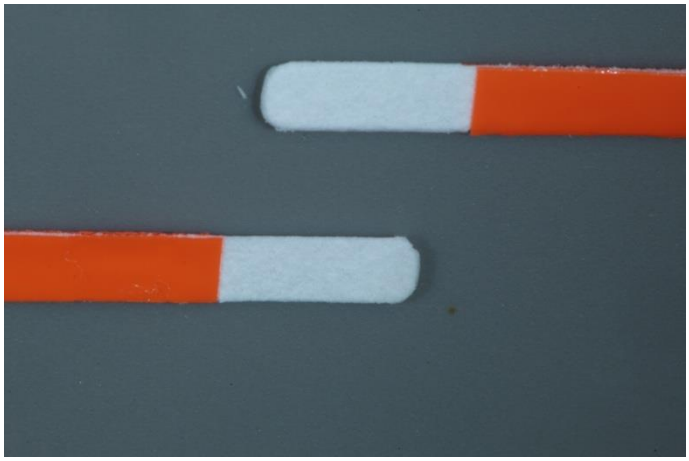


FIGURE 49 - Close up of the Periopaper adsorbent paper for the PCF and PICF extraction cytokines method. The white part enters the sulcus while the orange (wax) part is held by the pliers. In this extraction method, the orange wax part is cut and discarded.

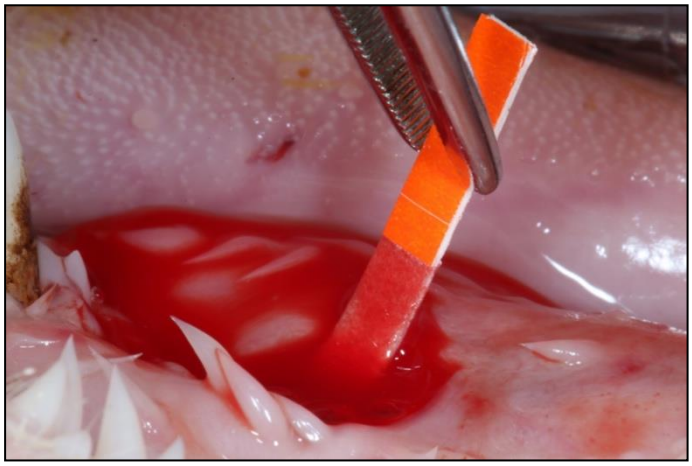


FIGURE 50 - Blood sample extraction at the time of first incision. Immediately after incision a periopaper strip is placed in the center of incision to collect blood.

FIGURE 51 - Close-up of the Periopaper® adsorbent paper for the periodontal (PCF) cytokinesextraction method. The paper was placed in the sulcus for 20 seconds. Notice the gingival health of natural teeth.

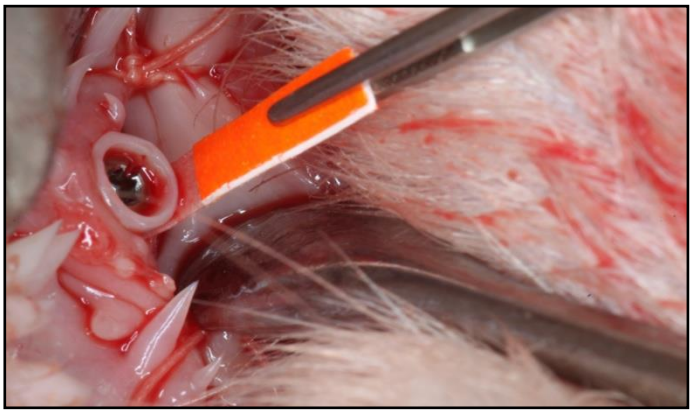


FIGURE 52 - Close-up of the Periopaper adsorbent paper for peri-implant (PICF) cytokines extraction method. (Acrylic Abutment). The paper was placed in the sulcus for 20 seconds. Notice the exudate emerging from the surgical wound.

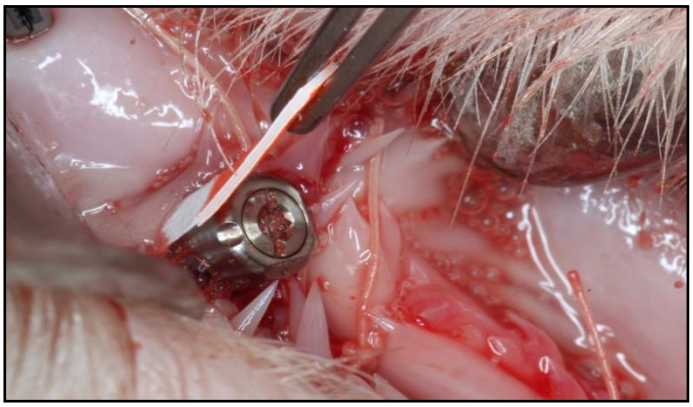


FIGURE 53 - Close-up of the Periopaper® adsorbent paper for peri-implant (PICF) cytokines extraction method. (CAD-CAM Titanium Abutment). The paper was placed in the sulcus for 20 seconds. Notice the exudate that emerges from the surgical wound

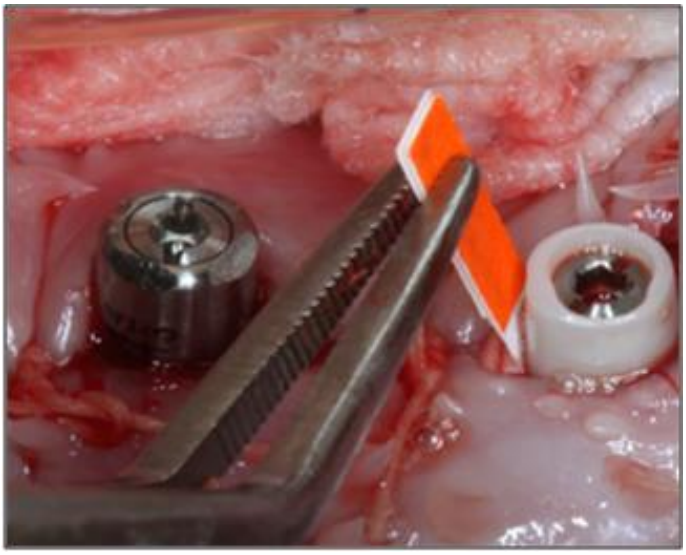


FIGURE 54 - Close-up of the Periopaper® adsorbent paper for peri-implant (PICF) cytokines extraction method. (Zirconia Abutment). The paper was placed in the sulcus for 20 seconds. Notice the exudate that emerges from the surgical wound.

3.2.7. PICF and PCF Cytokines Extraction Method from Biological Sheep Tissues at T1 and T3

T1 (one week) corresponded to a point where osseointegration was in the active phase while at T3 it corresponded to the conclusion of the osseointegration process.

The inflammatory collection sample protocol was the same for both. Two sheep were analyzed at T1 and another 4 were analyzed at T3.

The abutment was first rinsed with air and water, and isolated with cotton rolls, following which a periopaper® tip was placed in the implant sulcus for 20 second to discharge the initial exudate.

After 20 seconds a 1 mm Periopaper ® tip was inserted into the sulcus until a slight resistance was felt for 20 seconds. Four strips (4) were placed in an Eppendorf tube.

The method is shown on fig. 55 to 59.

FIGURE 55 - Close-up of inflammatory mediators in Periopaper® Extraction on a CAD-CAM acrylic abutment at T1/T3

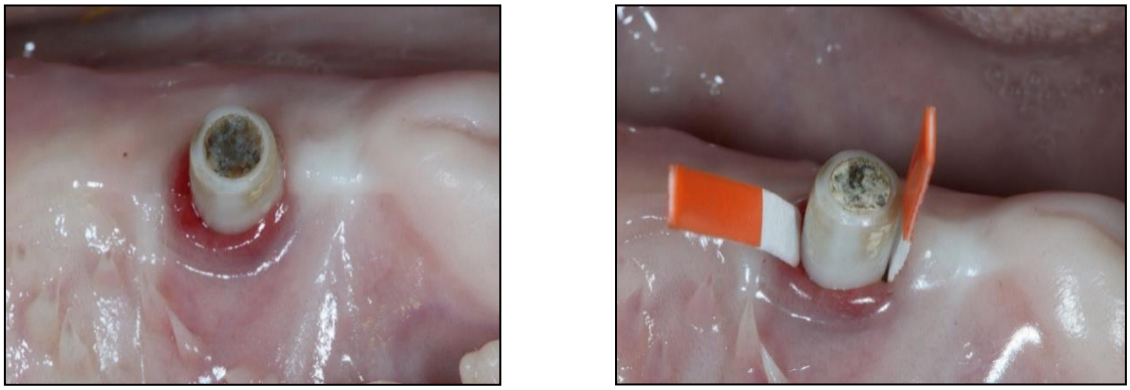


FIGURE 56 - Close-up of inflammatory mediators in Periopaper® Extraction on a CAD-CAM zirconia abutment at T1/T3

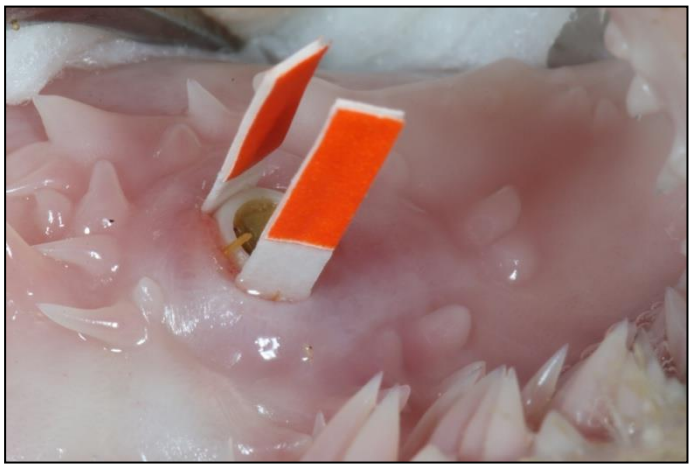


FIGURE 57 - Close-up of inflammatory mediators in Periopaper Extraction on a CAD-CAM acrylic abutment at T1/T3

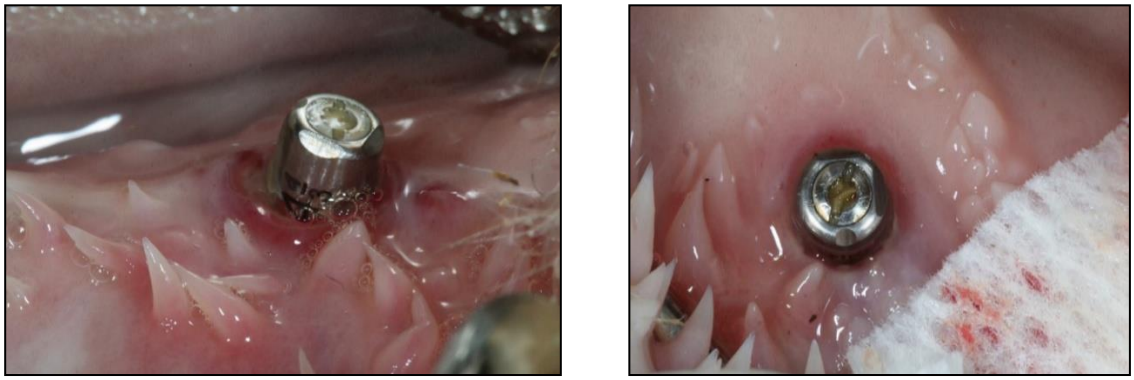


FIGURE 58 - Close-up of inflammatory Status before cytokine extraction methods on a titanium abutment at T1/T3

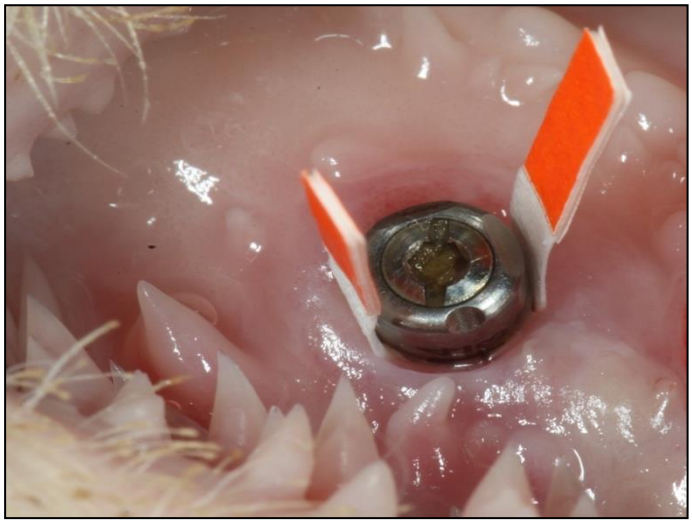


FIGURE 59 - Close-up of inflammatory mediators in Periopaper Extraction on a CAD-CAM titanium abutment at T1/T3

3.2.8. Sample Transportation Methodology

All samples were brought from the harvest location to the storage location embedded in dry ice and were stored to -80 degrees at IST (Instituto Superior Técnico) for all the experimental work. (fig. 60)

FIGURE 60 - Sample transportation in dry ice from research location in Santarém to sample readings at Instituto Superior Técnico**.**

3.2.9. Processing Samples and ELISA protocol

Each ELISA plate is composed of 96 wells which can be read independently in rows of 8. (each time frame is represented with R in the tables)

FIGURE 61 - All biological samples are stored and kept at - 80 degrees at the Instituto Superior Técnico. The temperature is monitored by means of a digital thermometer in the freezer base. Opening and closing is strictly supervised by the engineer responsible.

All ELISA reagents were brought to room temperature (18-25℃) before use and stored at -20ºC after each experiment. (fig. 61)

Preparation followed the aliquots procedure dilutions for the calibration curve. Inflammatory reaction measurements were based entirely on ELISA concentration principles.

The Elisa kits were all checked (Elabscience®) (fig. 62) for their intended use which, in this protocol, was to detect IL-1β and IL6 ovid (sheep) concentrations.

FIGURE 62 - ELISA kits for Sheep IL-1β and IL6.

Test principles were followed according to manufacturer specifications (Elabscience®).

The experiments were done in columns of 8 wells, but the samples were not read all at the same time. In some cases, 2 columns were selected and in others more columns were read., In doing so, it allowed us to control the extraction method and the calibration curve.

Most importantly, each time the ELISA cytokine reading was set, independently of the number of columns made, the protocol described below was strictly adhered to.

3.2.10. Elisa Essay Methodology

The micro ELISA plate provided in the kit had been pre-coated with an antibody specific to the IL-1β or 6 (depending on the kit).

This kit recognizes natural and recombinant Sheep IL-1β/IL6. No significant cross-reactivity or interference between Sheep IL-1β and analogues was observed. If the Elisa reaction is positive, then it is certain that the Interleukin

intended is present. The detection range in the kit used for this protocol is 31.25-2000 pg/mL for IL-1β and 78.125-5000 pg/mL for IL6.

3.2.11. Step-By-Step Elisa Procedures (short resume)

FIGURE 63. Lab Material for ELISA reading.

In the first step, standards or samples were added to the appropriate micro ELISA plate wells and combined with the specific antibody. The Second step, a biotinylated detection antibody specific to the desired IL and Avidin-Horseradish Peroxidase (HRP) conjugate is added to each microplate well in succession and incubated. Free components were washed away with a Buffer Solution. In the third step the substrate solution was added to each well.

Only those wells that contain the desired IL, biotinylated detection antibody and Avidin-HRP conjugate appeared blue in color.

In the final step, the enzyme-substrate reaction was terminated with the addition of a sulphuric acid solution and the color turned yellow.

The optical density (OD) was measured spectrophotometrically at a wavelength of 450 nm ± 2 nm. The OD value was proportional to the concentration of the desired IL.

3.2.12. Standard Preparation for Calibration Curve

Standards are pre-prepared, known concentrations of the antibody that each manufacturer delivers along with the ELISA kit in order to produce a calibration curve. (fig. 64)

The objective was to have a calibrated graphic (calibration curve) so the concentrations of experimental samples can be read.

Standards were prepared 15 minutes or less before use, by means of a centrifuge at 10,000 rpm for 1 minute and reconstituting the Standard solution with 1.0mL of Standard Reference and Sample Diluent.

The manufacturer recommends tightening the lid, and letting it stand for 10 minutes and turning it over several times.

After it dissolves fully it is mixed thoroughly with a pipette. This reconstitution produced a stock solution of 5000pg/ml.

After this procedure, serial dilutions were then produced as needed (serial dilution in the wells directly is not permitted by the manufacturer). The recommended concentrations of the ELISA plates were as follows:

For IL6 5000、2500、1250、625、312.5、156.25、78.125、0 pg./ml. and for

IL-1β 2000、1000、500,250,125、62.5、31.25、0 pg./ml

FIGURE 64 - ELISA kits for IL-1β and IL6 inflammatory cytokine quantification. Reagents are represented on the left and the ELISA plate on the right.

3.2.13. Extraction method and Reagent preparation

Each time an experiment is staged it includes removal of the sample from the -80ºC chamber and the reagents from the -20ºC freezer.

Periopaper® was cut in two with scissors leaving the wax part off, with only the white paper part remaining for cytokines extraction. (fig. 65 and 66)

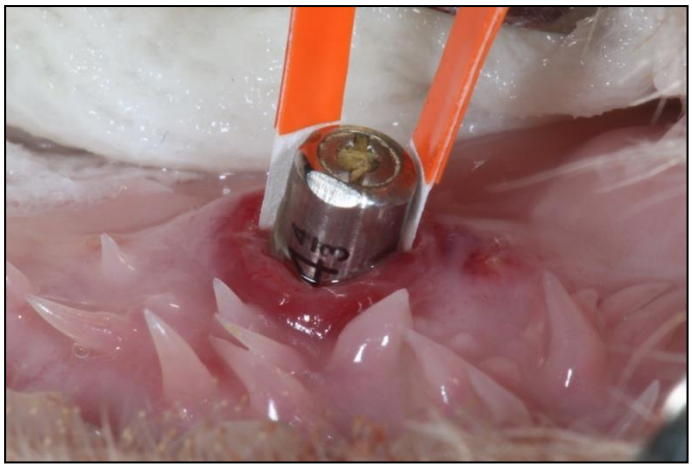
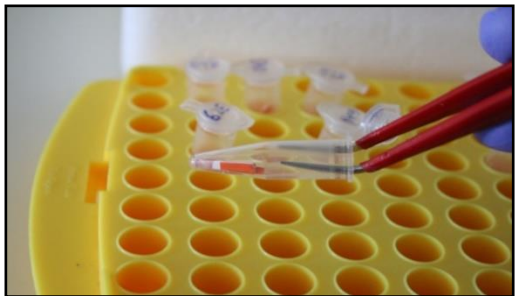


FIGURE 65 - Close up of the periopaper® in the cytokine extraction protocol. Notice that the white part is in the sulcus while the orange wax isn't in contact with anything. The wax part was cut off leaving only the white adsorbent part. This is to ensure that there was no bias in the the extraction protocol.



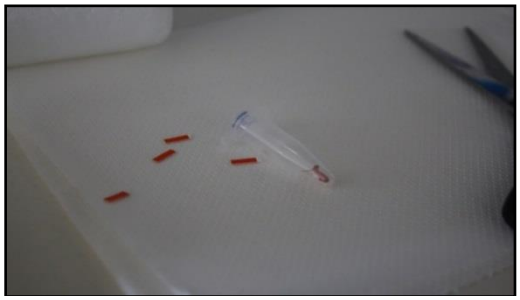


FIGURE 66 - Close up of Eppendorf tube preparation for cytokine reading.

The white paper part was then placed in an Eppendorf tube with 200 ml of coated buffer solution and was left on ice for 30 minutes. (fig. 67)

FIGURE 67 - Extraction protocol means leaving it on ice after buffer solution was placed for 30 minutes

It was placed in a centrifuge after extraction for 10 minutes at 5º degrees Celsius at 12.000 RPM. (fig. 68)

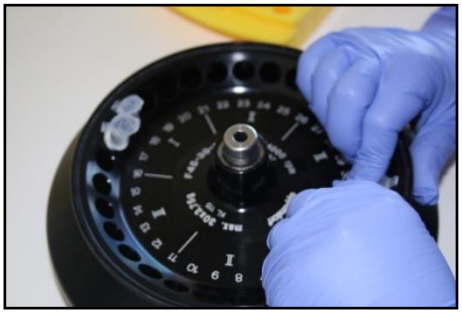


FIGURE 68 - Close up of sample placement in a centrifuge

100µL of Standard, Blank, or Sample was added per well. Reference Standard and Sample Diluent Biotinylated Detection Ab were added to the blank well.

The liquid was then removed from each well and left unwashed. (fig. 69)

100µL of Biotinylated Detection Ab working solution was immediately added to each well.

FIGURE 69 - Close up of extracted samples in a centrifuge

The plate sealer was covered, and the plate carefully taped to ensure thorough mixing and incubated for 1 hour at 37°C. (fig. 70)

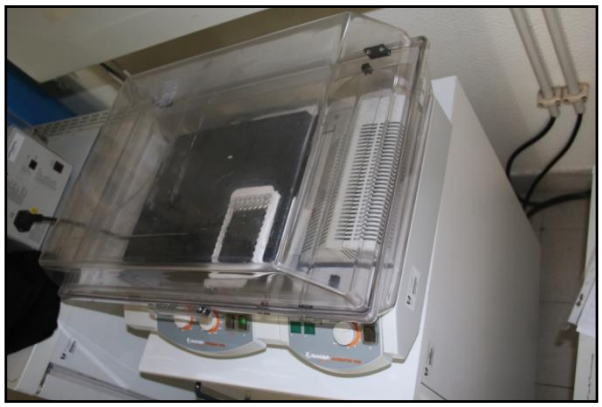


FIGURE 70 - The figure on the left shows the ELISA mixer and the on the right the controlled temperature room at 37º Celsius is shown.

Each well was aspirated and washed, repeating the process three times. Washing was done by filling each well with washing buffer (approximately 350µL) with a multi-channel pipette. Following this the liquid was removed from the Elisa wells at each step.

After the last wash, the remained washing buffer was removed by aspirating or decanting. the plate was turned over and tapped against thick clean absorbent paper.

100µL of HRP Conjugate working solution was added to each well, covered with the plate sealer and set to Incubate for 30 minutes at 37°C.

The washing process was repeated five times. (fig. 71)

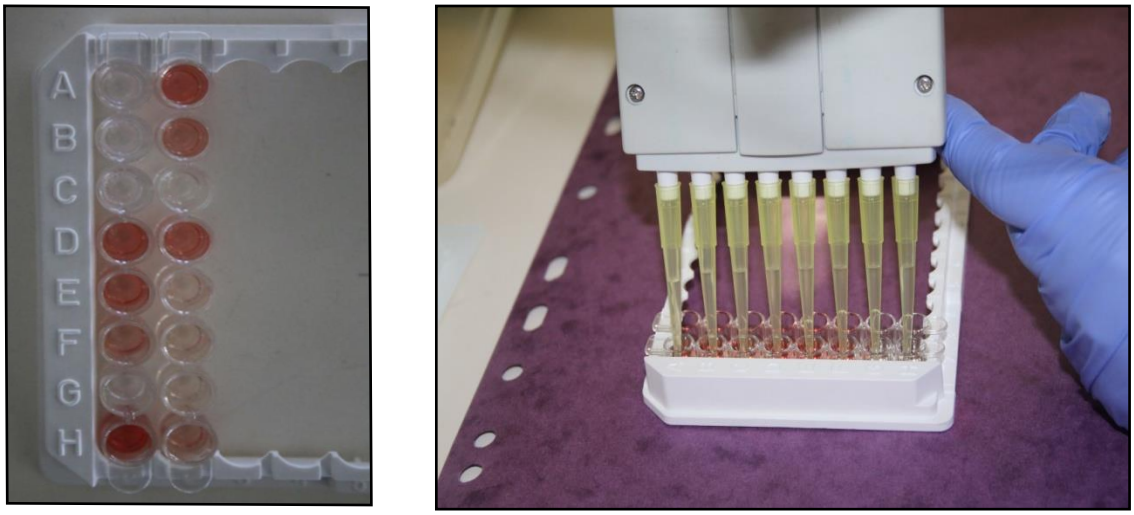


FIGURE 71 - Multichannel pipette in the washing phase. On the left is the Elisa plate before reagent and on the right, the washing buffer solution pipette.

90µL of Substrate Solution was added to each well after washing and covered with a new plate sealer and left to incubate for approximately 15 minutes at 37°C, with the plate protected from light at all times. The reaction time could be shortened or extended according to the actual color change, but not by more than 30 minutes.

In our experiment all the samples were taken at 25 min.

When apparent gradient appeared in standard wells, the reaction was terminated.

Finally, 50µL of Stop Solution was added to each well, turning the the color yellow immediately. The well order to add the stop solution was the same as the substrate solution. (fig. 72)

FIGURE 72 - Elisa plate after stop solution was added. Notice the first row, corresponding to the calibration curve points: more yellow means more concentration of IL.

3.2.14. Sample reading

The optical density (OD value) of each well was determined immediately using a micro-plate reader set to 450 nm. The micro-plate reader was opened in advance, the instrument pre-heated, and the testing parameters.

3.2.15. Calculation of results

Both for the standards of the calibration curve and the interleukin samples, the duplicate and triplicate readings were averaged. And for each standard and sample, the average zero standard optical density was subtracted. In doing so, a standard curve was created by plotting the mean OD value for each standard on the y-axis against the concentration on the x-axis and a best fit curve drawn through the points on the graphic. In the software interface Excel, a best fitting equation of standard curve was calculated using OD values and concentrations of standard sample. The software calculated the concentration of samples after entering the OD value of the samples. If samples were diluted, the concentration calculated from the standard curve was multiplied by the dilution factor. If the OD of the sample exceeded the upper limit of the standard curve, it was retested after appropriate dilution. The actual concentration was calculated and if thought necessary the concentration was multiplied by the dilution factor.

3.2.16. Statistical Methodology

To relate quantitative variables (such as IL6, IL-1β inflammation) to qualitative. variables (such as the material or moment, T0, T1 or T3), the following procedure was undertaken:

When there were two cases (moment) in the qualitative variable (moment), the parametric test T was used if the quantitative variable had a normal distribution or the samples in each of the two groups were large (more than 30). If any of these assumptions were not verified, then the non-parametric alternative Mann-Whitney test was used.

When the qualitative variable had 3 or more cases (material), the parametric ANOVA test was used if the quantitative variable had a normal distribution or

the samples in each of the groups was large (more than 30), and there was homogeneity in the variances. If the assumptions were not verified, then the nonparametric alternative Kruskall-Wallis test was used.

For all tests in this study the significance level of 5% ($p \leq 0,05$) was considered stastistically significant.

SECTION 3.3 RESULTS OF ANIMAL EXPERIMENTAL MODEL

3.3.1. Results of Calibration Curve for IL6 and IL-1β

3.3.1.1. For IL6

The calibration curve was done by reading the standards. Calibration points were read in duplicates and in some samples in triplicates, representing each point as an average with a standard deviation. In the IL6 and IL-1β of the animal study there is almost a linear proportion when measuring optical densities and concentrations.

For IL6 the optimum Excel equation that serves the calibration curve was y=0,0005x+0,0686R^2 = 0,99897 and from that, all the concentration points were calculated.

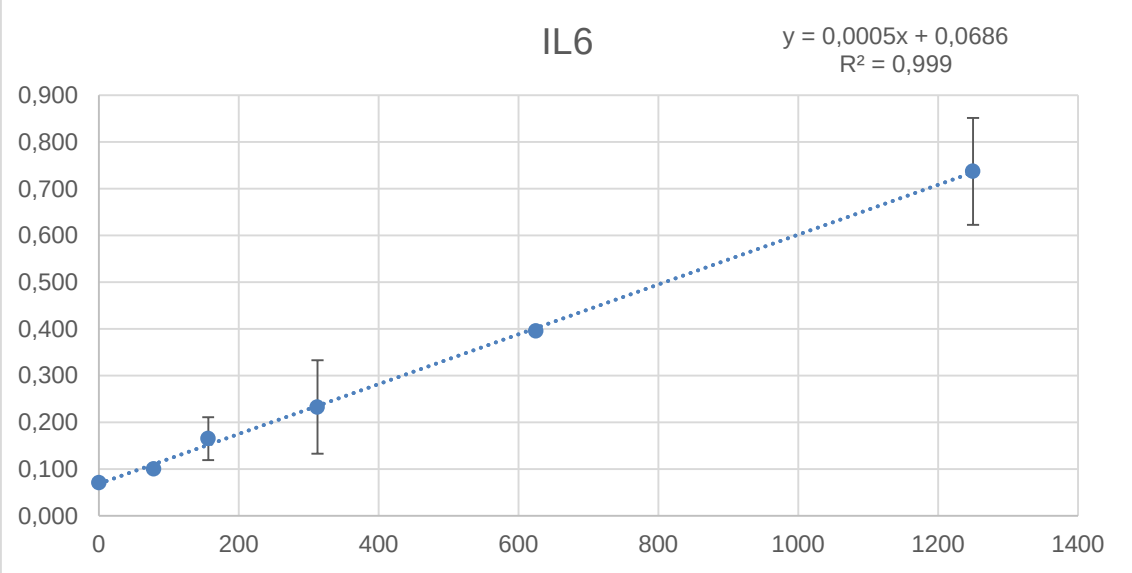


FIGURE 73 - Calibration Curve for IL6 interleukin

Table 2 - IL6 Standards results. Calibration curve concentration points measured at different time frames.

[]	R1	R2	R3	R4	R5	M	SD
5000	2,108	2,337				2,223	0,115
2500	1,013					1,013	0,000
1250	0,637				0,837	0,737	0,100
625	0,403	0,448			0,336	0,396	0,046
312,5	0,234				0,231	0,233	0,002
156,25	0,165					0,165	0,000
78,13	0,105	0,104	0,09	0,108	0,095	0,100	0,007
0	0,071					0,071	0,000

[]- Concentration R- time frame where the sample was read. M- Mean SD- Standard Deviation

3.3.1.2. For IL-1β

The best equation that serves the curve for IL-1β was y = 0,0012x + 0,0596 R^2 = 0,99626 and from that all the sample concentration points were calculated.

Table 3 represents each time frame (R) in which the duplicate and triplicates samples were done.

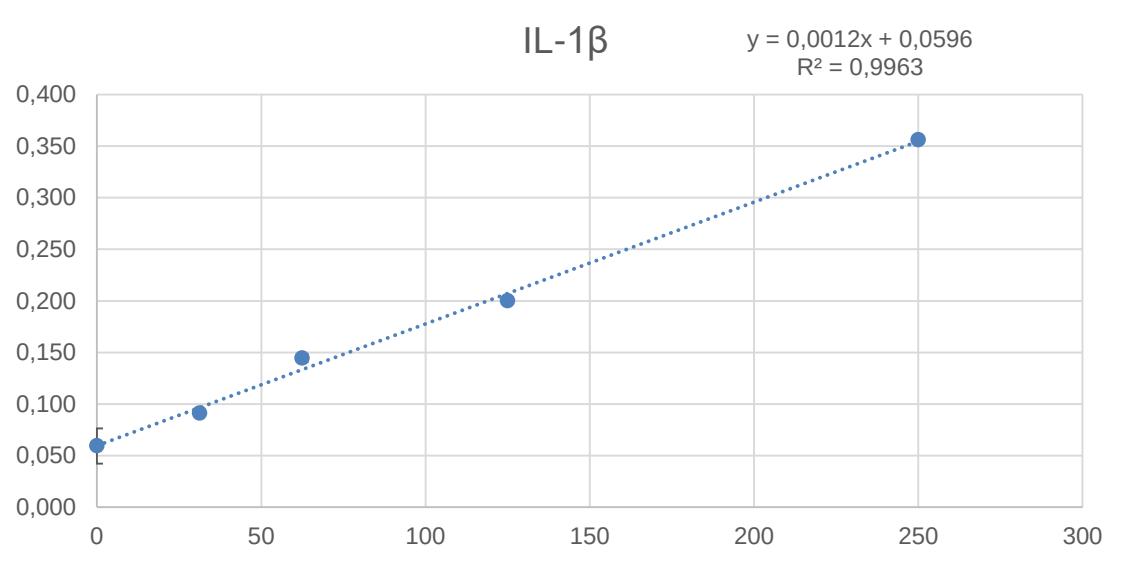


FIGURE 74 - Calibration Curve for IL-1β interleukin

Table 3 - Standards IL-1β Calibration curve concentration points measured at different time frames.

[]	R1	R2	R3	R4	M	SD
2000	1,28				1,280	0,000
1000	1,038				1,038	0,000
500	0,576			0,584	0,580	0,004
250	0,356				0,356	0,000
125	0,217		0,183		0,200	0,017
62,5	0,144	0,176	0,131	0,127	0,145	0,019
31,25	0,066	0,103	0,081	0,115	0,091	0,019
0	0,057	0,056	0,065		0,059	0,004

[]- Concentration R- time frame where the sample was read. M- Mean SD- Standard Deviation

3.3.2. Interleukin IL6 and IL-1β Results

The results were divided by: 1- Interleukin, 2-By time frame (BL, T1 and T3) and 3-By Material (Z, A and T).

3.3.2.1. IL6 inflammatory performance results:

3.3.2.1.1. IL6 inflammatory behavior of different materials (Z, A, T) at Baseline T0 period

At baseline all sheep were subjected to extraction samples (R1) and some were randomly duplicated (R2) and triplicated (R3).

For all extraction time frames, the right side of the mandible was used for IL-1β extraction data and the left side of the mandible sheep for IL6 data extraction.

The summary of the inflammatory results are shown in table 4. The results are by sheep number and by abutment placed over different time frames (R).

Table 4 - IL6 Baseline T0 results by sheep in pg/ml

Sheep Nº/Material	R1	R2	R3	Mean
1T	41			41,00
1Z	0		9	4,50
1A	5		0	2,50
2T	0		33	16,50
2Z	17		11	14,00
2A	5		21	13,00
3T	17		81	49,00
3Z	87		51	69,00
3A	25			25,00
4T	11		11	11,00
4Z	33			33,00
4A	23			23,00
5T	53			53,00
5Z	69			69,00
5A	33			33,00
6T	65	55		60,00
6Z	85	23		54,00
6A	79	59		69,00

R1- Sample Reading time frame R2-Duplicate R3-Triplicates T-Titanium Z-Zirconia A-Acrylic

The different results obtained at T0 for IL6 are displayed in the fig. 75.

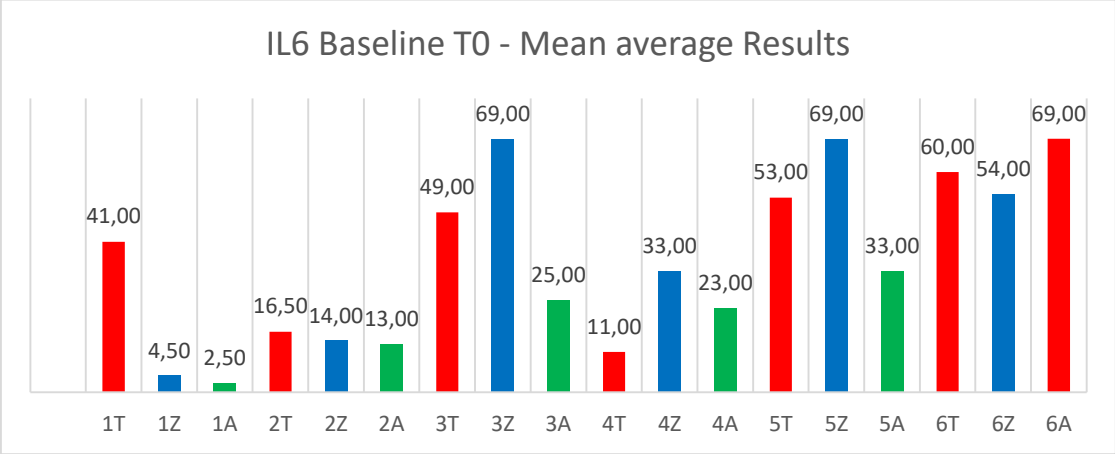


FIGURE 75 - IL6 Concentration in pg/ml of Interleukins at T0 (baseline). Zirconia, Acrylic and Titanium Healing abutment (Z, A, T) IL6 interleukin display. Red- CAD-CAM, Titanium, Blue CAD-CAM Zirconia, Green-CAD-CAM Acrylic

The overall IL6 results found on the T0 moment are shown in table 5.

Table 5 - Mean and standard deviation of Concentration in pg/ml of Interleukins at T0 (Baseline)

Material	IL6 pg/ml	IL-1β pg/ml
Z	38 ± 32	6 ± 5
A	28 ± 26	10 ± 13
T	37 ± 27	10 ± 14

3.3.2.1.2. IL6 inflammatory behaviour of different materials (Z, A, T) at one month (T1)

The summary of the inflammatory results for T1 are shown in table 6. The results are by sheep number and by abutment placed over different time frames (R1) and duplicates (R2)

At T1 sheep number 5 and 6 were analyzed, and duplicates were made, and an average calculated.

Table 6 - IL6 inflammatory concentration levels at T1. Different concentrations in pg/ml were read in different time frames (R).

Sheep Nº/Material	R1	R2	Mean
1T			
1Z			
1A			
2T			
2Z			
2A			
3T			
3Z			
3A			
4T			
4Z			
4A			
5T	35		35
5Z	0	41	21
5A	21		21
6T	19		19
6Z	7	27	17
6A	0	63	32

R1- Sample Reading at each time frame R2-Duplicates: T-Titanium Z-Zirconia A-Acrylic

The different results obtained at T1 for IL6 are shown in fig. 76.

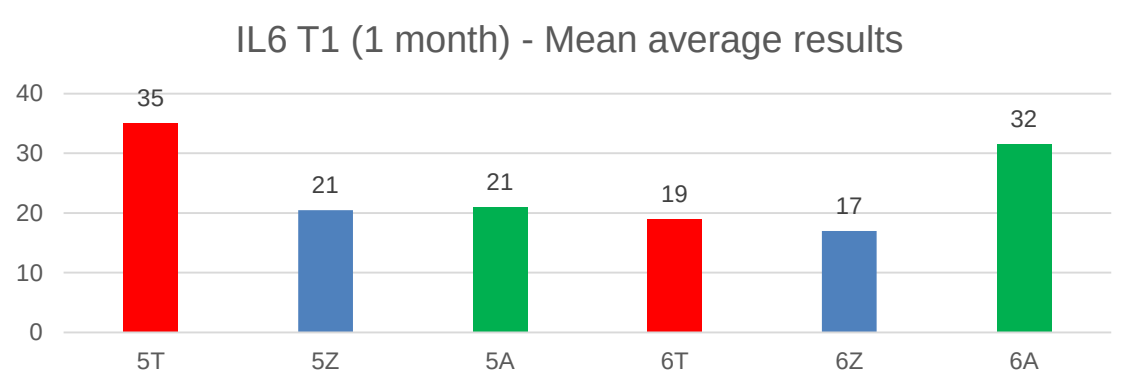


FIGURE 76 - Concentration in pg/ml of Interleukin 6 at T1 (1month). Zirconia, Acrylic and Titanium Healing abutment (Z, A, T) IL6 interleukin display.

The overall IL6 results found at the T1 moment are shown in table 7.

Table 7 - Mean and standard deviation of Concentration in pg/ml of Interleukins at T1 (1 month)

Material	IL6 pg/ml	IL-1β pg/ml
Z	19 ± 19	11± 0
A	28 ± 32	2 ± 3
T	27 ± 11	14± 12

3.3.2.1.3. IL6 inflammatory behavior of different materials (Z, A, T) at 3 months T3

The summary of the inflammatory results for T3 are shown in table 8. The results are by sheep number and by abutment placed over different time frames (R).

At T3 four implants were lost and thus not entered for final statistical evaluation, represented by L in the table 8.

Table 8 - IL6 concentration at T3 time frame. pg/ml

Sheep Nº/Material	R1	R2	Mean
1T	15		15
1Z	25		25
1A	33		33
2T	L	L	L
2Z	L	L	L
2A	L	L	L
3T	39	91	65
3Z	41	61	51
3A	53	153	103
4T	17	49	33
4Z	L	L	L
4A	35	91	63
5T			
5Z			
5A			

6T

6Z

6A

R1- Sample Reading R2- Duplicate Sample Reading L-Lost implant T-Titanium Z-Zirconia A-Acrylic

The different results obtained at T3 for IL6 are presented in fig. 77.

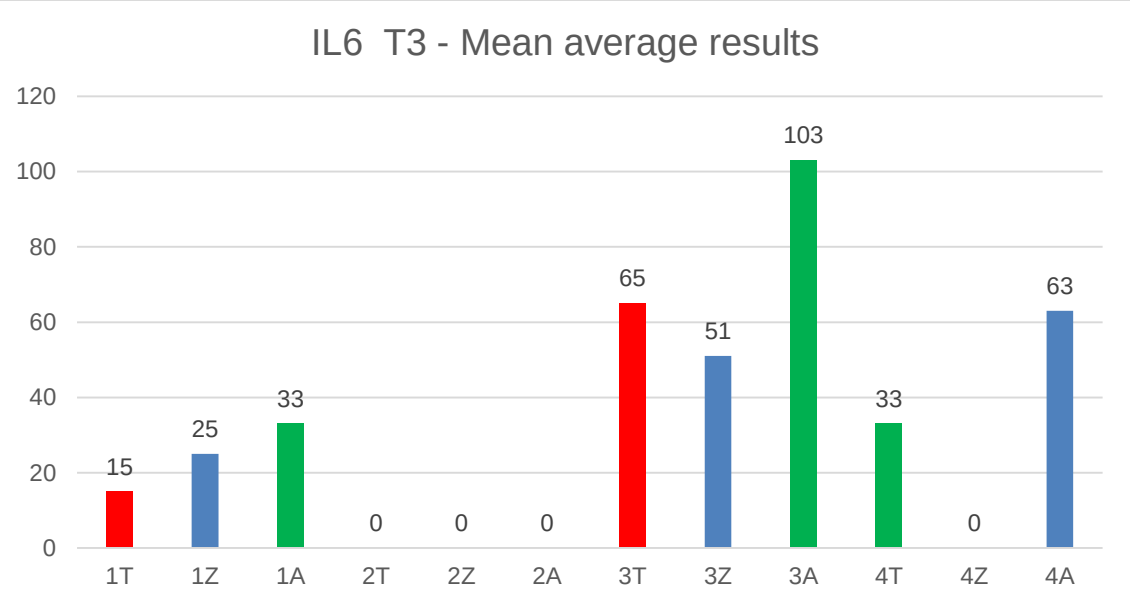


FIGURE 77 - IL6 Concentration in pg/ml of Interleukins at T3 (3 month). Zirconia, Acrylic and Titanium Healing abutment (Z, A, T).

The overall IL6 results found at the T3 moment are shown in table 9.

Table 9 - Mean and standard deviation of concentration in pg/ml of Interleukins at T3 (3 month)

Material	IL6 pg/ml	IL-1β pg/ml
Z	42 ± 18	8 ± 9
A	73 ± 50	19 ± 16
T	57 ± 31	13 ± 12

3.3.2.1.4. Statistical evaluation of the results at T0, T1 and T3

To verify, at each time frame (T0, T1 and T3), if there were significant differences between the different biomaterials (Z, A, T), the Kruskall-Wallis parametric test was used, since there was no normal distribution in the IL6 variable in all groups (small samples).

For each time, the *p*-values were, respectively, 0,597, 0,497 and 0,481.

Which were all above the level of significance. Therefore, in each time frame (T0, T1 and T3), the differences in IL6 between the different materials were not significant. (table 10).

Table 10 - Hypothesis and statistical conclusions of the Behavior of different materials (Z, A, T) in each time frame T0, T1, T3. Interleukin 6

Time Frame	H, L, S*	Test	Null Hypothesis	P-Value
T0	S	Kruskall-Wallis	Retain	0,597
T1	S	Kruskall-Wallis	Retain	0,497
T3	S	Kruskall-Wallis	Retain	0,481

*H-Higher L-Lower S-Same

3.3.2.1.5. IL6 Inflammatory behavior of different materials over time (from Baseline T0 to three-month T3)

After seeing the behavior of the interleukins in each time frame we also wanted to know, the behavior over time from implant placement to 3 months.

The summary of the inflammatory results for T0, T1 and T3 by biomaterial used, are shown in fig. 78.

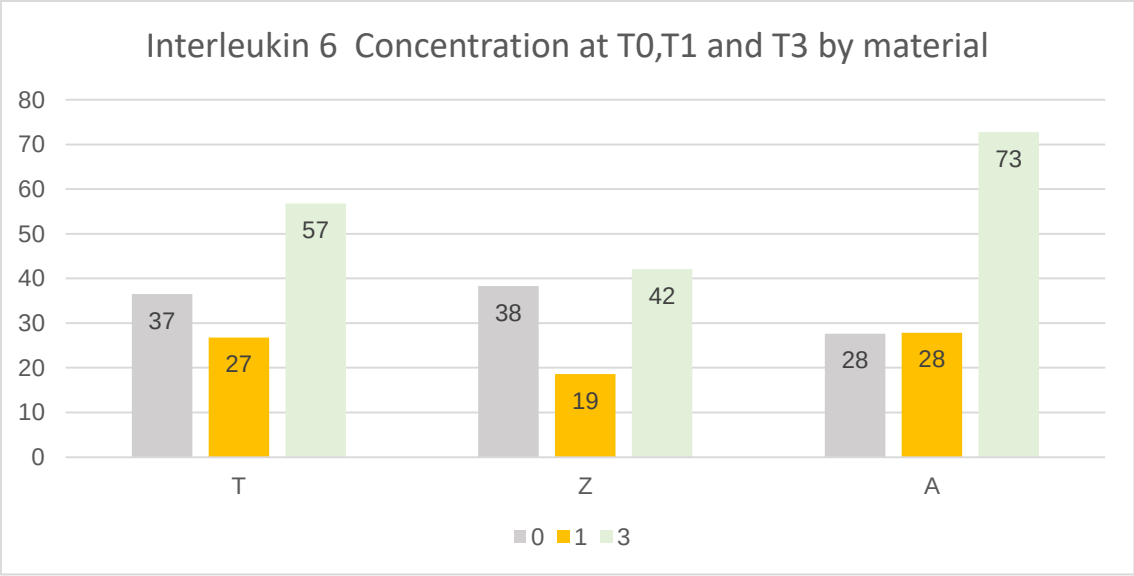


FIGURE 78 - IL6 Concentration in pg/ml of Interleukins at T0, T1 and T3 (by material). Zirconia, Acrylic and Titanium Healing abutment (Z, A, T) display

The different results obtained at T0 and T3 for Z, A and T (for IL6) are displayed in fig. 79.

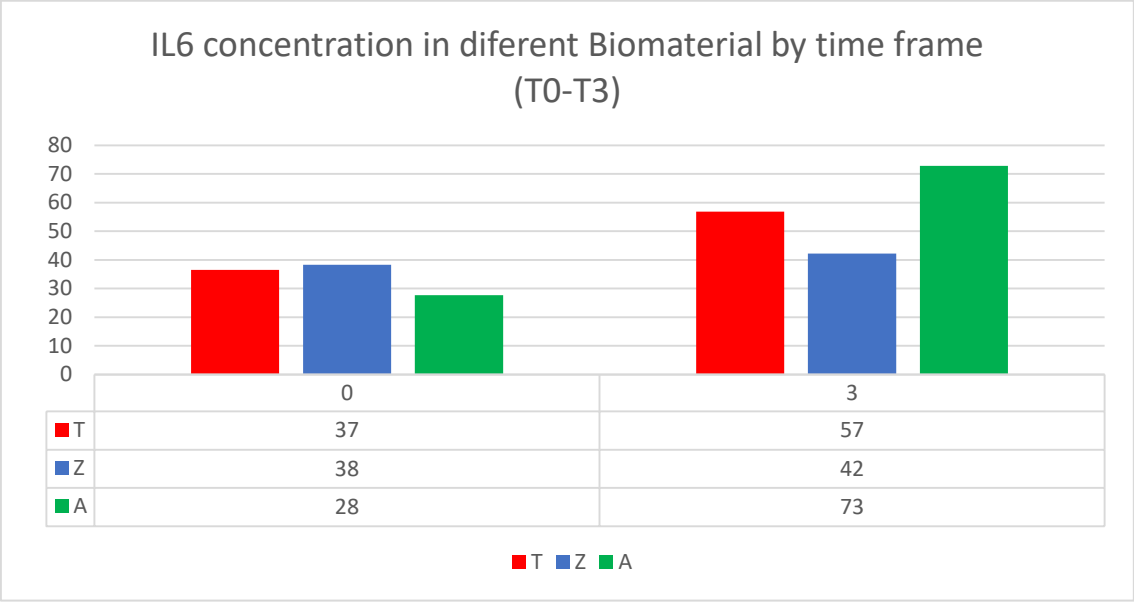


FIGURE 79 - IL6 Concentration in pg/ml of Interleukins at T0 and T3. Zirconia, Acrylic and Titanium Healing abutment (Z, A, T) IL6 and interleukin display

IL6 graphically displayed interleukin variation from T0 to T3.

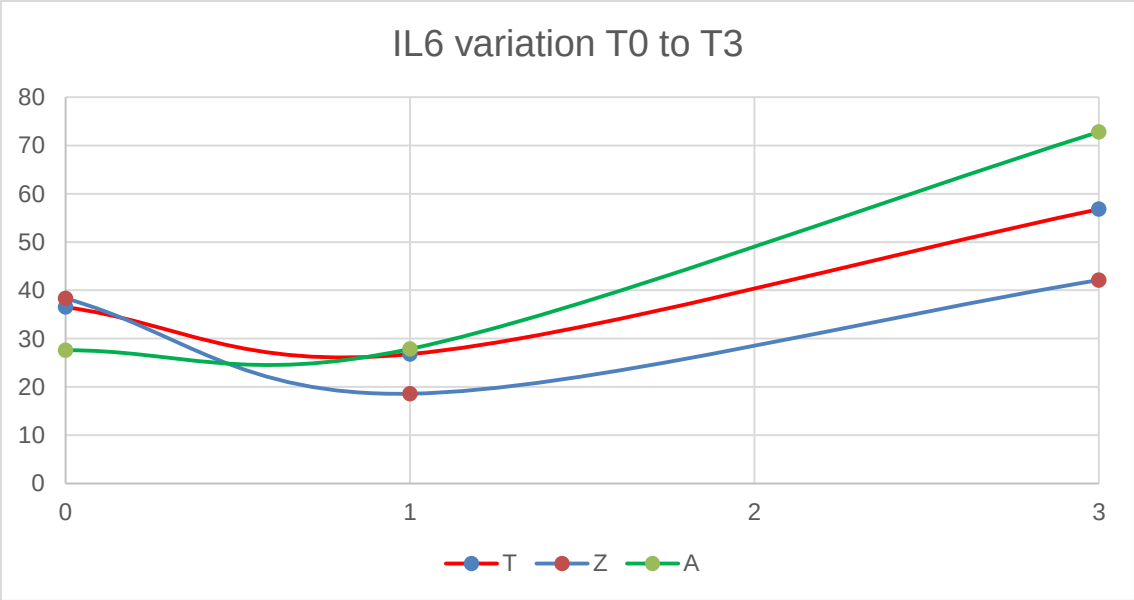


FIGURE 80 - IL6 Concentration in pg/ml of Interleukins at T0, T1 and T3. Zirconia, Acrylic and Titanium Healing abutment (Z, A, T). IL6 shows a pattern of increased concentrations from T0 to T3

The aim was to verify for each material, Titanium, Zirconium and Acrylic, if there were significant differences between T0 and T3. For this, the non-parametric Mann-Whitney test was used, since the variable, in each material, IL6 had no normal distribution in both groups (T0 and T3, small samples).

For each material, the *p*-values were, respectively, 1,000; 0,857; 0,095. Therefore, in each material, the differences of IL6 between T0 and T3 were not significant. (table 11)

Table 11 - Hypothesis and statistical conclusions of the Behavior of Titanium, Acrylic and Zirconia between T0 and T3. Interleukin 6

Time Frame	H, L, S*	Test	Null Hypothesis	P-Value
A	S	Mann-Whitney	Retain	0,095
T	S	Mann-Whitney	Retain	1,000
Z	S	Mann-Whitney	Retain	0,857

*H-Higher L-Lower S-Same

3.3.2.1.6. Compare Peri-implant Inflammatory Levels (IL6) with Sheep blood levels (BL) at baseline T0

Another measure of comparison is to compare the inflammatory response around peri-implant tissues against the basal blood levels at the time of surgery.

This represents a control group since the blood sample will tell us the basal inflammatory status of the sheep before implant placement.

At time T0, for each material, the IL6 value was compared with blood levels (BL), whose basal value averaged 71,689 pg/ml. For this, the nonparametric signal test (equivalent to a non-parametric binomial test) was used. This option was due to the samples not being normally distributed and therefore the T-test (small samples) was not able to be performed.

For each material, at T0, IL6 there were significantly lower blood values (all *p*-values gave 0.031 <0.05). (table 12).

Table 12 - Hypothesis and statistical conclusions of the Behavior of interleukin 6 on PICF and blood Fluid (BF) at T0

Time Frame	H, L, S*	Test	Null Hypothesis	P-Value
Z	L	Related-Samples Sign test	Reject	0,031
T	L	Related-Samples Sign test	Reject	0,031
A	L	Related-Samples Sign test	Reject	0,031

*H-Higher L-Lower S-Same T-Titanium Z-Zirconia A-Acrylic

3.3.2.1.7. Compare Peri-implant Inflammatory Levels (IL6) with Sheep Periodontal Crevicular Fluid (PCV) at baseline T0 and T3

Another control group compared peri-implant interleukin levels with periodontal levels to see if the reaction to an alloplastic material resembled the periodontal response.

Samples of periodontal fluids were taken at two different time frames, one at T0 baseline and another at T3, 3 months later.

The goal was to compare peri-implant fluids at T0 with periodontal fluid also at T0 and for T3.

The inflammatory levels of IL6 at baseline are represented in a table together with the PCF.

The averages found between PICF IL6 with PCF IL6 are shown in table 13.

Table 13 - Mean and standard deviation of Concentration in pg/ml of Interleukins at in Z, A, T at T0 and T3 and in Periodontal crevicular fluid (T0 and T3) and Blood Fluid (BF) at T0

Material	IL6 pg/ml	IL-1β pg/ml
Z T0	38 ± 32	6 ± 5
A T0	28 ± 26	10 ± 13
T T0	37 ± 27	10 ± 14
Z T3	42 ± 18	8 ± 9
A T3	73 ± 50	19 ± 16
T T3	57 ± 31	13 ± 12
PCF at T0	29	6
PCF at T3	39	3
BF at T0	72	16

The results obtained for PCF at T0 and T3 for Z, A and T (for IL6) are displayed in fig. 81, Blood samples are also displayed in the same graphic.

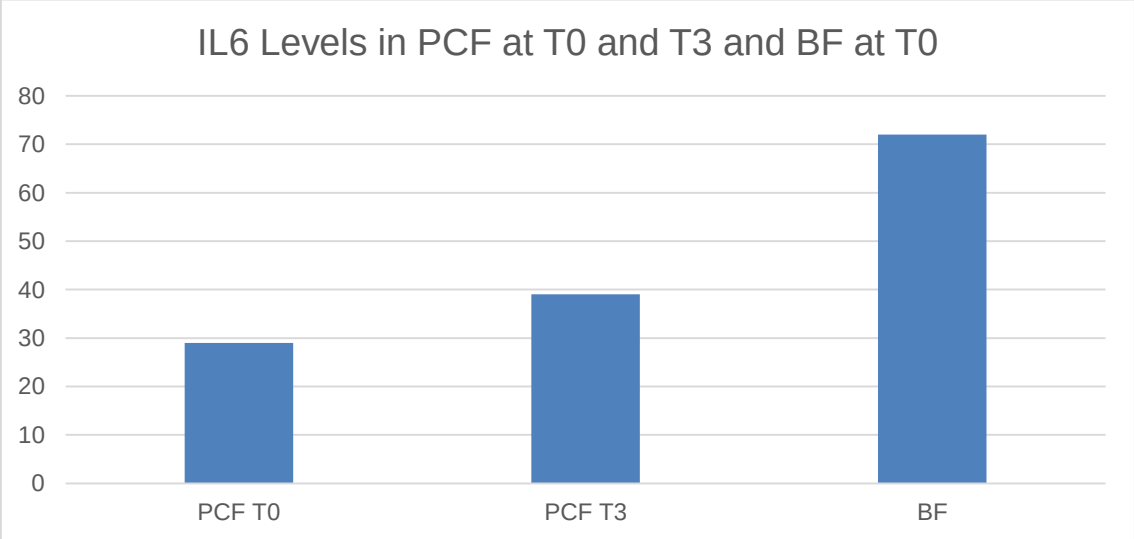


FIGURE 81 - Concentration in pg/ml of PCF Interleukin 6 at T0 and T3 and (BF) blood fluids at T0.

The comparison of different time frames with different abutments and the total PCF (independently of time) and blood levels are displayed in table 13.

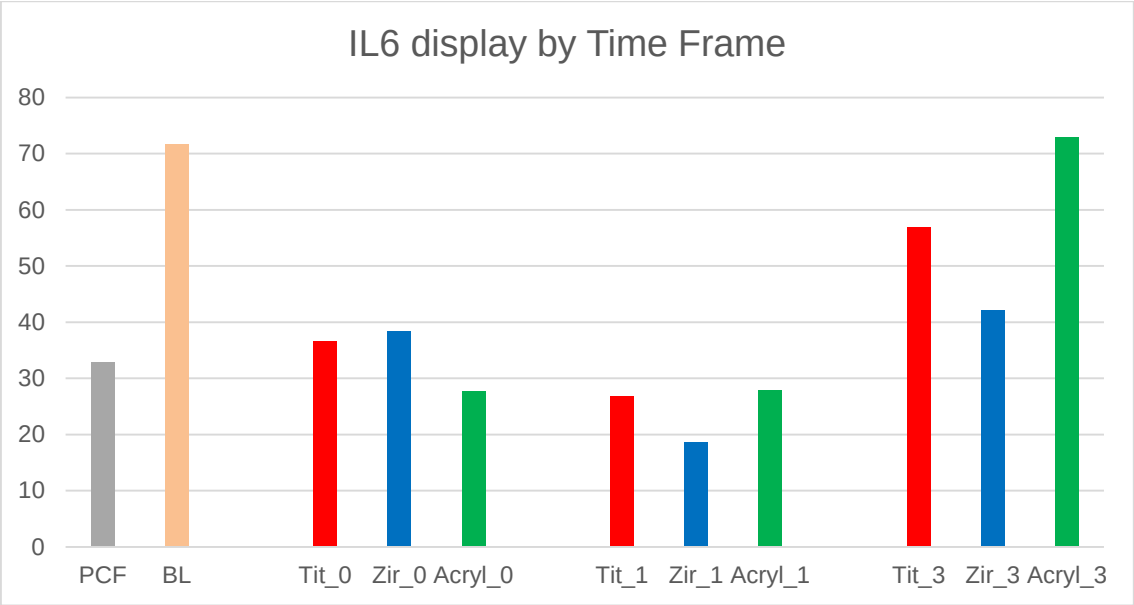


FIGURE 82 - IL6 Concentration in pg/ml of Interleukins by time frame (T0, T1 and T3). Zirconia, Acrylic and Titanium Healing abutment (Z, A, T). Periodontal crevicular fluid (PCF) and blood levels (BF) fluids are also present

3.3.2.1.8. PCF Basal Values (Baseline T0) compared to PICF at T0

At Baseline T0, for each material (Z, A, T), the value of IL6 with initial PCF, was 29 pg/ml (average). The non-parametric signal test (equivalent to a non-parametric binomial test) was used. This option is due to the samples not being normally distributed, ruling out the T-test (small samples).

All materials at T0, IL6 were not significantly lower or significantly higher than the initial PCF tooth value (*p*-values gave 0,688> 0.05), that is, there were no significant differences between IL6, at T0, with initial PCF (T0). (table 14)

Table 14 - Hypothesis and statistical conclusions of the behavior of interleukin 6 on PICF and Periodontal Crevicular Fluid (PCF) at T0

Time Frame	H, L, S*	Test	Null Hypothesis	*P*-Value
Z	S	Related-Samples Sign test	Retain	0,688
T	S	Related-Samples Sign test	Retain	0,688
A	S	Related-Samples Sign test	Retain	0,688

*H-Higher L-Lower S-Same T-Titanium Z-Zirconia A-Acrylic

3.3.2.1.9. AS 3 Month Values comparing PICF with PCF

For each material at time T3, the value of IL6 with final PCF, was 39 pg/ml (average). For this purpose, the nonparametric signal test (equivalent to the non-parametric binomial test) was used. This option is due to the samples not being normally distributed ruling out the use of the T-test (small samples).

For any material, at T3, IL6 is not significantly lower or significantly higher than the value of the final tooth (*p*-values gave 1,000> 0,05), that is, there are no significant differences between IL6 at T3 with final PCF (T0). (table 15)

Table 15 - Hypothesis and statistical conclusions of the Behavior of interleukin 6 on PICF and Periodontal Crevicular Fluid (PCF) at T0

Time Frame	H, L, S*	Test	Null Hypothesis	P-Value
Z	S	Related-Samples Sign test	Retain	1,000
T	S	Related-Samples Sign test	Retain	1,000
A	S	Related-Samples Sign test	Retain	1,000

*H-Higher L-Lower S-Same T-Titanium Z-Zirconia A-Acrylic

3.3.2.2. AS IL-1β Results

3.3.2.2.1. IL-1β Interleukin behavior in different materials (Z, A, T) at each time frame (Baseline T0)

The summary of the IL-1β inflammatory results are shown in table 16. The results are by sheep number and by abutment placed in different time frames (R).

Table 16 - IL-1β Baseline concentrations at T0 in different time frames.

Sheep Nº/Material	R1	R2	Mean
1T	15		15
1Z	0		0
1A	3		3
2T	42	7	25
2Z	9		9
2A	4		4
3T	0		0
3Z	0	1	1
3A	0	0	0
4T	0	1	1
4Z	5		5
4A	0		0
5T	6		6
5Z	15	9	12
5A	30	29	30
6T	10		10
6Z	7		7
6A	1	12	7

R- sample collected at different time frames R2-Duplicates T-Titanium Z-Zirconia A-Acrylic

The different results obtained at T0 for IL-1β are shown in fig. 83.

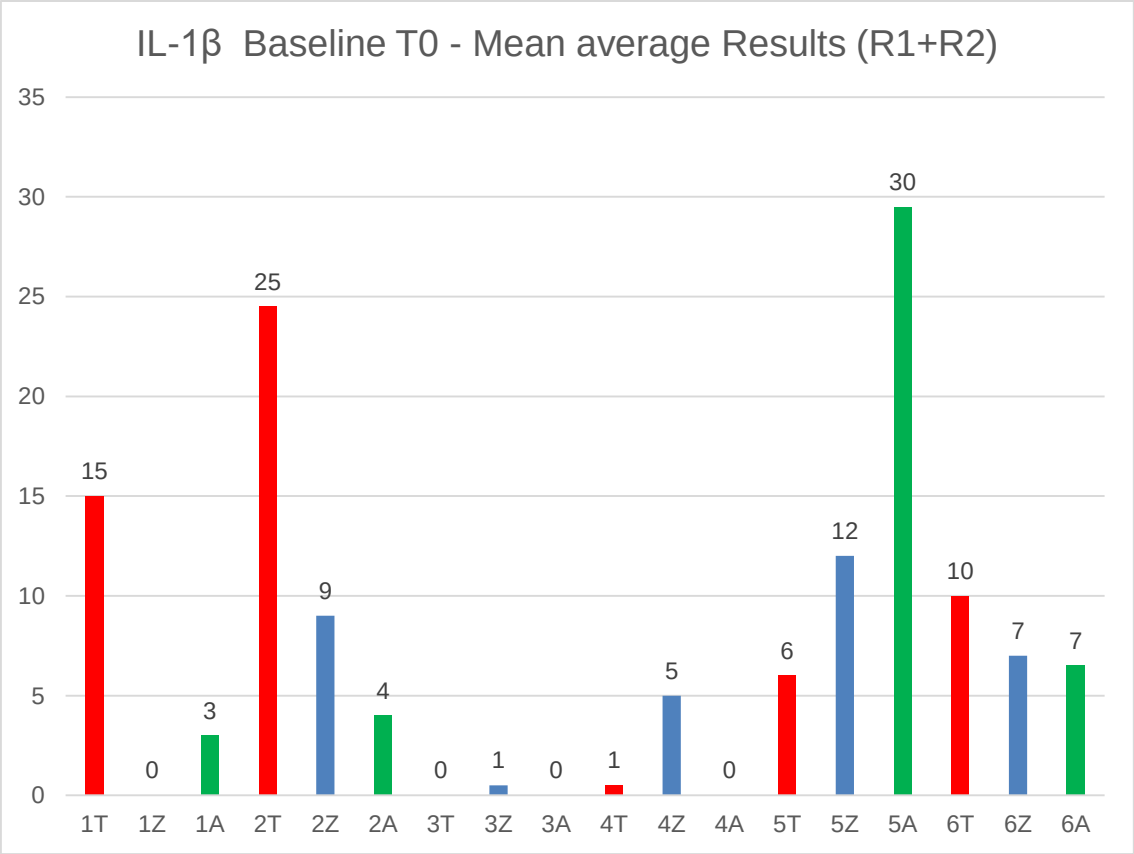


FIGURE 83 - IL-1β Concentration in pg/ml of Interleukins at T0 (baseline). Zirconia, Acrylic and Titanium Healing abutment (Z, A, T) IL-1β display. Red-CAD-CAM, Titanium, Blue-CAD-CAM Zirconia, Green-CAD-CAM Acrylic

Overall IL-1β results registered at the T0 moment are shown in table 17.

Table 17 - Mean and standard deviation of Concentration in pg/ml of Interleukins at T0 (Baseline)

Material	IL6 pg/ml	IL-1β pg/ml
Z	38 ± 32	6 ± 5
A	28 ± 26	10 ± 13
T	37 ± 27	10 ± 14

3.3.2.2.2. Behavior of IL-1β in different materials (Z, A, T) at each time frame (T1 one month)

The summary of the IL-1β inflammatory results are exposed in table 18. The results are by sheep number and by abutment placed at different time frames (R).

Table 18 - IL-1β concentrations in pg/ml at T1

Sheep Nº/Material	R1	R2	Mean
1T			
1Z			
1A			
2T			
2Z			
2A			
3T			
3Z			
3A			
4T			
4Z			
4A			
5T	28	20	24
5Z	11		11
5A	5		5
6T	0	8	4
6Z	11		11
6A	0		0

R- sample collected at different time frames R2-Duplicates T-Titanium Z-Zirconia A-Acrylic

The different results obtained at T1 for IL-1β are displayed in fig. 84.

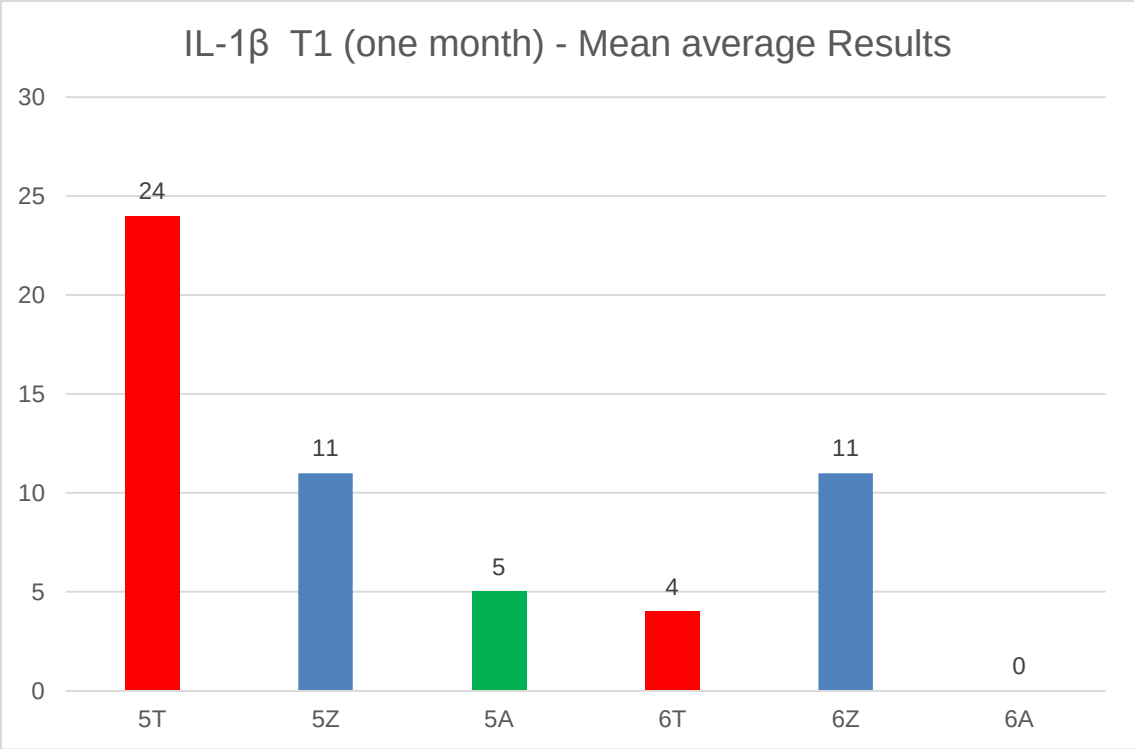


FIGURE 84 - Concentration in pg/ml of IL-1β at T1 (1month). Zirconia, Acrylic and Titanium Healing abutment (Z, A, T) IL-1β display

3.3.2.2.3. IL-1β behavior of different materials (Z, A, T) in each time frame (3 Month)

The summary of the IL-1β inflammatory results are exposed in table 19. The results are by sheep number and by abutment placed at different time frames (R). In this analysis 2 implants were lost, represented by the letter L in the chart.

Table 19 - IL-1β concentration levels in pg/ml at T3

Sheep Nº/Material	R1	R2	Mean
1T	2		2
1Z	0		0
1A	5		5
2T	L		L
2Z	3		3
2A	40	2	21
3T	15	30	22,5
3Z	8	20	14
3A	38	21	29,5
4T	8		8
4Z	L		L
4A	24	5	14,5
5T			
5Z			
5A			
6T			
6Z			
6A			

R- sample collected at different time frames R2-Duplicates T-Titanium Z-Zirconia A-Acrylic

The different results obtained at T3 for IL-1β are displayed in fig. 85.

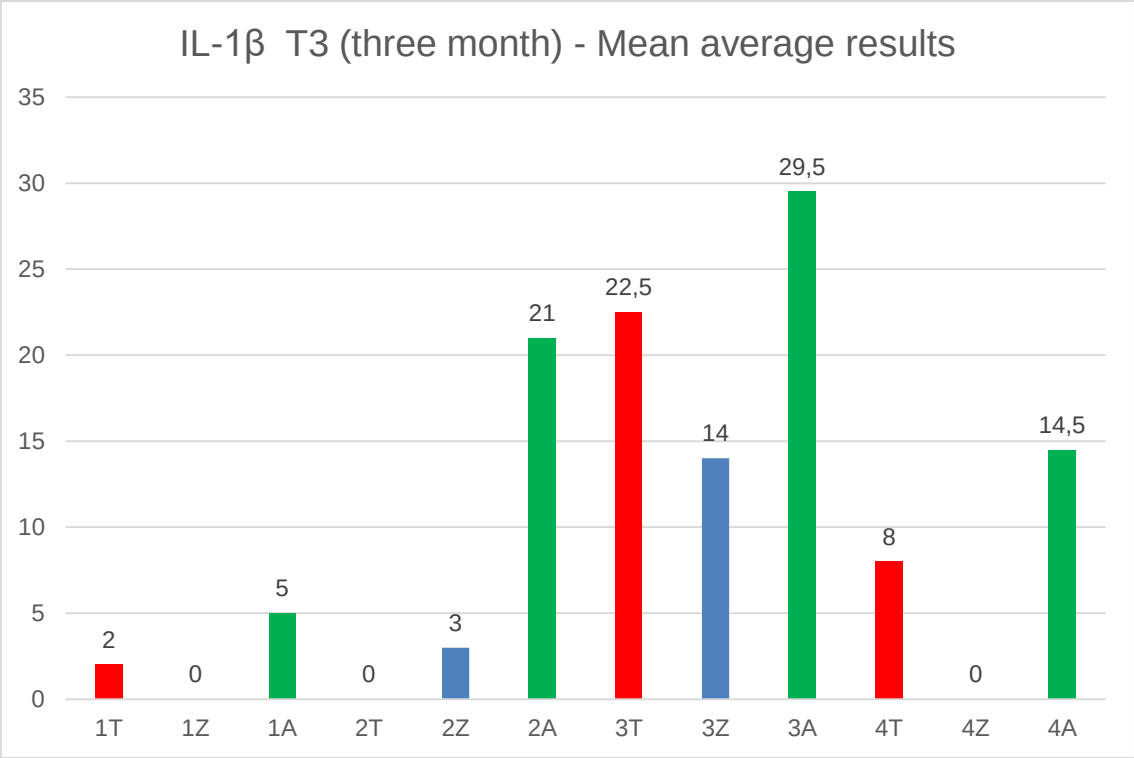


FIGURE 85 - Concentration in pg/ml of IL-1β at T3 (3month). Zirconia, Acrylic and Titanium Healing abutment (Z, A, T) IL-1β interleukin display

3.3.2.2.4. IL-1β Statistical evaluation of the Results at T0, T1 and T3

In order to verify, if there were significant differences between the different materials at each time (T0, T1 and T3), the Kruskall-Wallis parametric test was applied, as the IL-1β variable was not normally distributed in all groups (small samples).

For each time, the *p*-values were, respectively, 0,857; 0,357 and 0,237. All were above the level of significance. Therefore, at each time, the differences of IL-1β between the different materials were not significant.

Table 20 - Hypothesis and statistical conclusions of the Behavior of different materials (Z, A, T) in each time frame T0, T1, T3. IL-1β

Time Frame	H, L, S*	Test	Null Hypothesis	*P*-Value
T0	S	kruskall-Wallis	Retain	0,857
T1	S	kruskall-Wallis	Retain	0,357
T3	S	kruskall-Wallis	Retain	0,237

*H-Higher L-Lower S-Same

3.3.2.2.5. IL-1β Behavior of Different materials over time (from Baseline T0 to 3 month T3)

The same protocol that was used for study IL6, was also used for IL-1β and so the behavior of the different Interleukins was studied to see and compare the variation over time and by material.

The summary of the IL-1β inflammatory results for T0, T1 and T3 by biomaterial used, are exposed in fig. 86.

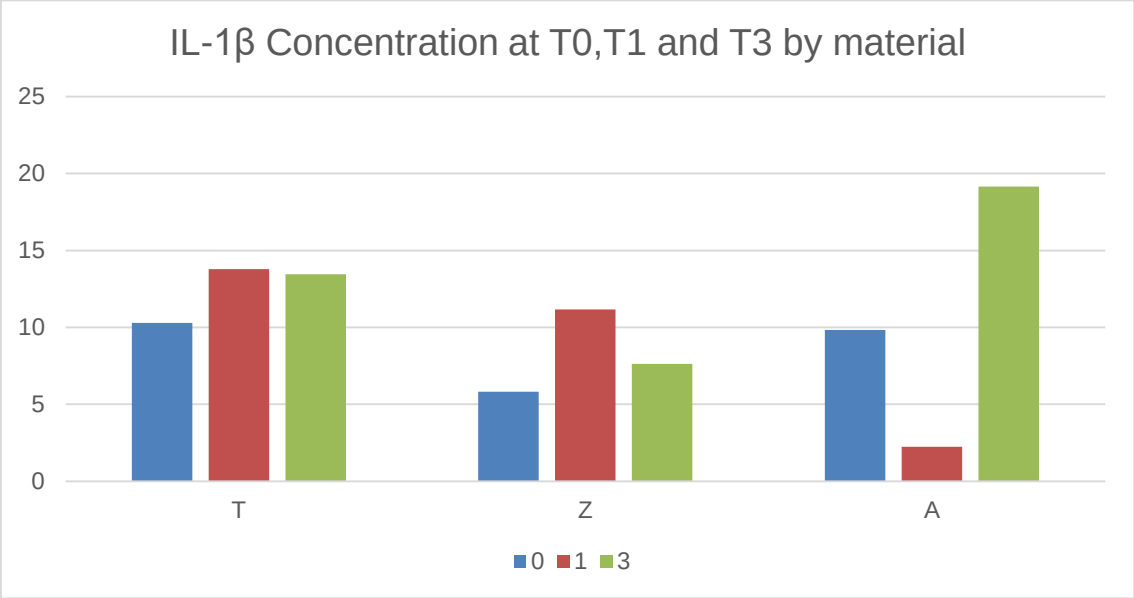


FIGURE 86 - IL-1β Concentration in pg/ml of Interleukins at T0, T1 and T3 (by material). Zirconia, Acrylic and Titanium Healing abutment (Z, A, T) display

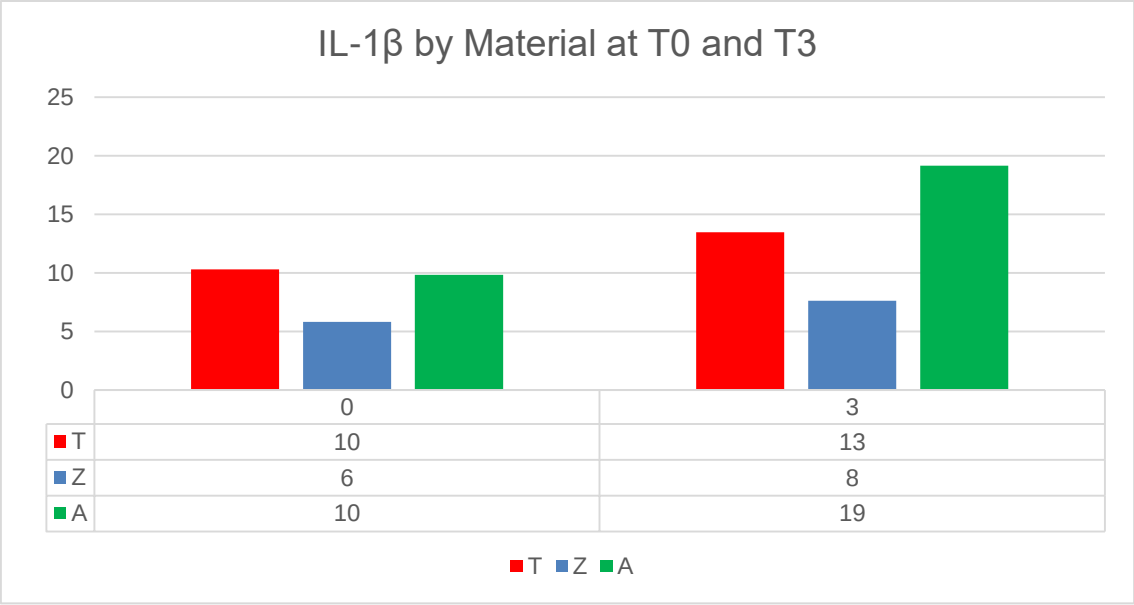


FIGURE 87 - Concentration in pg/ml of Interleukins IL-1β at T0 and T3. Zirconia, Acrylic and Titanium Healing abutment (Z, A, T) IL-1β and interleukin display

IL-1β illustrates graphically interleukin variation from T0 to T3.

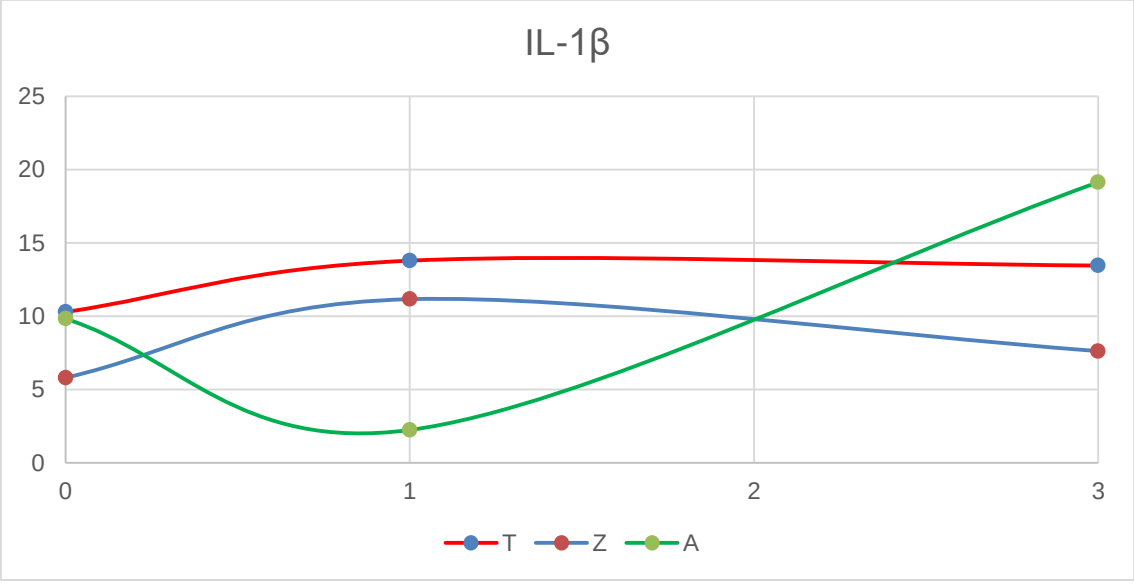


FIGURE 88 - Concentration in pg/ml of Interleukins at T0, T1 and T3. Zirconia, Acrylic and Titanium Healing abutment (Z, A, T). IL-1β shows a pattern of a small increased concentrations from T0 to T3

For each material, Titanium, Zirconium and Acrylic, for confirmation that there were or there were not significant differences between T0 and T3, the non-

parametric Mann-Whitney test was used, since the variable in each material IL-1β were not normally distributed in both groups (T0 and T3, small samples).

For each material, the *p*-values were, respectively, 1,000; 0,905; 0,286. Therefore, in each material, the differences in IL-1β between T0 and T3 were not significant.

Table 21 - Hypothesis and statistical conclusions of the Behavior of Titanium, Acrylic and Zirconia between T0 and T3. IL-1β

Time Frame	H, L, S*	Test	Null Hypothesis	*P*-Value
A	S	Mann-Whitney	Retain	0,286
T	S	Mann-Whitney	Retain	1,000
Z	S	Mann-Whitney	Retain	0,905

*H-Higher L-Lower S-Same T-Titanium Z-Zirconia A-Acrylic

3.3.2.2.6. Compare Peri-implant Inflammatory Levels (IL-1β) with Sheep blood levels (BL) at baseline T0

As in IL6 the experimental samples were compared with the control groups: the blood samples for sheep characterization and the periodontal sulcular fluid to contrast them with natural teeth.

The summary of the IL-1β inflammatory results are shown in table 22. The results are compared with BL at the time of incision.

Table 22 - IL-1β concentrations in PCF at T0 and T3 and BF

PCF T0	PCF T3	BF T0
0	3	19
8		20
9		15
		9
		15
		29
		6

At time T0, for each material, the IL-1β value was compared with the blood sample, with a value of 16,2857 pg/ml (average). For this, the nonparametric

signal test (equivalent to a non-parametric binomial test) was used. This option was chosen because the samples were not normally distributed, ruling out the use of the T-test (small samples).

For zirconia, at T0, IL-1β is significantly lower than the blood sample value (*p*-value 0,031 <0,05). However, for titanium and acrylic, *p*-value = 0,219> 0,05 and *p*-value = 0,375> 0,05. That is, at T0, with titanium and acrylic, it cannot be guaranteed that IL-1β is significantly lower for the blood sample (there were no significant differences between IL-1β, at T0, for the blood sample, for titanium and acrylic materials).

Table 23 - Hypothesis and statistical conclusions of the Behavior of IL-1β on PICF and blood Fluid (BF) at T0

Time Frame	H, L, S*	Test	Null Hypothesis	*P*-Value
Z	L	Related-Samples Sign test	Reject	0,031
T	S	Related-Samples Sign test	Retain	0,219
A	S	Related-Samples Sign test	Retain	0,375

*H-Higher L-Lower S-Same T-Titanium Z-Zirconia A-Acrylic

3.3.2.2.7. Compare Peri-implant Inflammatory Levels (IL-1β) with Sheep Periodontal Crevicular Fluid (PCV) at baseline T0

At time T0, for each material, the value of IL-1β with initial PCF, was 4 pg/ml (average). When we compare them, the non-parametric signal test (equivalent to a non-parametric binomial test) was used. This option is due to the samples not being normally distributed and thus ruling out the T-test (small samples).

For titanium, zirconia and acrylic, at T0, there were no significant differences between IL-1β, at T0, with initial PCF (*p*-values = 0,688; 0,688; 1,000, respectively> 0,05).

Table 24 - Hypothesis and statistical conclusions of the Behavior of IL-1β on PICF and Periodontal Crevicular Fluid (PCF) at T0

Time Frame	H, L, S*	Test	Null Hypothesis	*P*-Value
Z	S	Related-Samples Sign test	Retain	0,688
T	S	Related-Samples Sign test	Retain	0,688
A	S	Related-Samples Sign test	Retain	1,000

*H-Higher L-Lower S-Same T-Titanium Z-Zirconia A-Acrylic

3.3.2.2.8. AS 3 Month Values of PCF vs PICF at T3

At time T3, for each material, the value of IL-1β with final PCF, was 3 pg/ml (average). The non-parametric signal test (equivalent to a non-parametric binomial test) was used for comparison. This option is due to the samples are not being normally distributed and thys ruling out the T-test (small samples).

For any material, at T3, IL-1β is significantly lower or significantly higher than the value of the final tooth (*p*-values 1,000, 1,000 and 0,125> 0,05), that is, there are no significant differences between IL-1β at T3 with final PCF of a tooth, in any of the 3 materials.

Table 25 - Hypothesis and statistical conclusions of the Behavior of IL-1β on PICF and Periodontal Crevicular Fluid (PCF) at T0

Time Frame	H, L, S*	Test	Null Hypothesis	P-Value
Z	S	Related-Samples Sign test	Retain	1,000
T	S	Related-Samples Sign test	Retain	1,000
A	S	Related-Samples Sign test	Retain	0,125

*H-Higher L-Lower S-Same T-Titanium Z-Zirconia A-Acrylic

SECTION 3.4 DISCUSSION OF ANIMAL EXPERIMENTAL MODEL

The use of sheep as an experimental model in biomedical research has several applications in the field of medicine. (Meeusen et al. 2009)

In 1667, Frenchman Jean-Baptiste Denis performed the first-ever transfusion of blood from an animal to a human out of curiosity, transferring blood from a sheep to a 15-year old boy, both of whom survived the process.

This says a lot about the importance of sheep in experimental designs.

Mature sheep have 32 teeth, as do other ruminants. The front teeth in the lower jaw bite against a hard, toothless pad in the upper jaw. There is a large diastema between the incisors and molars making a reliable area to study endosseous implants as well as guided bone regeneration or trauma.

There are currently almost 2700 works using a sheep model in the literature and the percentage of papers which cite them varies between <1% and 8%. (Puc et al. 2018)

Sheep have been a source for interleukin studies in general medicine in a vast range of fields. (table 26)

Table 26 - Sheep Interleukins Experimental work in Medicine

Author	Year	Tissue - studied	IL studied
Kallapur	2011	Lung	IL1
Milligan	2017	Genitalia	IL-1β, IL8
Crespo	2013	White cell	IL4
Gossner	2013	Infection	IL4, IL5, IL13
Wolfe	2013	Amniotic fluid	IL8
Yan	2011	Intestine	IL1α, IL-1β, IL6, IL8
Hillman	2010	Lung	IL8, IL1
Dzidic	2010	White blood cells	IL1α, IL-1β, IL2, IL6
Zhu	2010	Placenta	IL18, IL6, IL8
Ingham	2008	Gut	IL10

For example to study inflammation at child birth and the consequences of a higher level of cytokines, a number of studies used the sheep model for stablish baselines. (Zhu et al. 2010; Wolfe et al. 2013)

In terms of the study of white blood cells, blood hemodynamics and auto immune response, the sheep model was used with very promising outcome results. (Dzidic et al. 2010)(Crespo et al. 2013)

Infection and microbiology is another field of research in interleukins, where the model has been extensively used and quoted. (Gossner et al. 2013)

Finally, there are some studies in literature in terms of gastrointestinal studys reportinging a cause–effect relationship in interleukins regulation.(Ingham et al. 2008; Yan et al. 2011).

As a result of ground-breaking studies carried out in the 1960s and 1970s, the baseline anatomy and physiology of this animal is well described.

In veterinary literature sheep diseases such as bluetongue, scrapie or visna (three of the major diseases that affect ovine cattle) interleukins and inflammatory levels have long been used to characterize the status of health and therapeutic induction.

Sheep are generally used in some forms of clinical testing, particularly to test certain transplantation medications, and surgery in general. Given the successful development of OVT73, sheep look to be promising candidates for future preliminary pharmaceutical testing.

In the dental field, particularly in oral surgery, there are some studies that clearly validate the sheep model for animal experiment. (Consolo et al. 2013; Yoo et al. 2014; Galli et al. 2015; Trisi et al. 2017; Jimbo et al. 2014)

In our study, we had to simulate intraorally a one stage implant placement with a healing abutment, so the diastema area of the sheep mandible was a candidate site that fulfilled all the pre-requisites.

One of the first alterations made to “traditional” human implant placement in the lower jaw, was to alter the position of the dental implants, an alteration to the standard implant placement parallel to adjacent teeth. In order to protect the work from the mandibular movement of the ruminant, the implants had to be

placed 4 mm below the crestal bone in a perpendicular line of adjacent teeth in order to engage the buccal and lingual cortex.

In doing so, not only are the implants protected from extensive grinding, but primary stability is also enhanced through the bicortical anchorage.

The incision line was performed according to the need to place the implant in that area.

Other authors prefer different locations in the sheep jaw such as the posterior mandibular angle or the lateral portion of the mandibular gonium under the masseter muscle or in other bones of the skull like the calvaria or parietal bone. (Trisi et al. 2017; Consolo et al. 2013; Jimbo et al. 2014).

The authors/investigators that place implants in those sites are mainly looking to study osseointegration healing processes, since those areas are well vascularized due to the presence of large quantities of trabecular bone.

Some studies opt for the iliac crest of the sheep to study bone apposition and behavior towards surface technology. (Yoo et al. 2014)(Trisi et al. 2016)

In our study, we didn't want to study bone to implant contact (BIC). Rather, we wanted to study autoimmune response. Thus, a situation that had a similar oral environment had to be created in order for the the abutments to stay in contact with the soft connective tissue, triggering an inflammatory response.

A total of 6 implants were lost in our study, between T1 and T3, a failure rate that is in accordance with the literature with comparable implant sites. (Trisi et al. 2017)

The objective of this study was to characterize inflammatory patterns and not histology research.

In choosing the sheep model we had the choice of several, pre-calibrated and validated biochemical/Interleukin kits available from a number of companys, that facilitated the search for specific immunological markers in biological fluids.

All the studies above mentioned used proven kits purchased from different companies such as Bio-Rad ™ or Tebu-bio ™. The Elisa kits are previous prepared to detect the antibody, with no cross-reactivity with other immune-cells present.

The fact that the kits are well proven, so if the immunoassay is positive then the results cannot have a false-positive or a false negative (or at least is rare, according to the manufacturer). In other words, there seems to be no cross-reactivity with other molecules.

The reaction antibody-antigen is only possible if the IL-1β or the IL6 is present in the peri-implant sulcus.

The kits were initially designed for detecting infection and inflammatory patterns in the ovine family. They are intended to detect large quantities of blood anomalies. The range of concentrations available in the peri-implant sulcus is much lower than blood or saliva concentrations, so a kit had to be chosen that detected Interleukin levels even at low concentrations.

The majority of the dental research that has studied biochemical markers uses periopaper® for sulcus collection.("Interleukin-1beta, Tumor Necrosis Factor-Alpha Levels and Neutrophil Elastase Activity in Peri-Implant Crevicular Fluid. - PubMed - NCBI" 2018; Ataoglu et al. 2002a; Baqui et al. 2000; Ataoglu et al. 2002b)

This is a proven technique as the adsorbent paper is regarded as the gold standard for sulcular biological fluid collection.

It is used for measuring the potential inflammatory reaction of tooth movement (Pramustika et al. 2018) in the orthodontic literature and the periodontal literature for intrasulcular fluid collection (Emecen-Huja et al. 2013) and is a well stablished technique in dental investigation.

This animal experiment gave us the opportunity to validate our extraction protocol, to see if the transportation methodology and the extraction cytokine protocol were correct.

The amount of fluid taken, was a concern, since all ELISA kits that test for ovine species are based on significant amounts of fluid or direct measurement of blood into the kit.

The extraction protocol for these types of biological fluids are well described in the literature, but the extraction protocol from a periopaper® strip taken from an ovine sheep is not.

The extraction methodology was first optimized at the IST, having the work of Emecen Huja (Emecen-Huja et al. 2013; Emecen-Huja, Hasan, and Miller 2015), as reference, where a technique of cutting the periopaper in two, leaving the wax part off is described, leading our team to use this protocol. To extract cytokines from the white adsorbent part it was left for 30 min in the buffer solution in ice before centrifugation. One could argue if that is enough time to extract the proteins, but the results showed consistent concentration values in every time frame.

The results show that the IL concentration varied over time and in each time frame IL6 and IL-1β, were present in the samples and were correctly extracted.

The results found are very uniform, at any point in time, where the material had an impact on the inflammatory (IL6/ IL-1β) levels, However, zirconia expressed significantly less IL than blood levels (control group) at T0, which can indicate a difference in early healing behavior.

At T0 clearly all the samples were very similar in interleukin concentrations. One possible interpretation is that the amount of cytokine released was related more strongly to the wound inflicted by the surgery itself, than by the biomaterial that was used.

Even with samples taken 1 hour after surgery was completed, the effect of trauma on the interleukin levels was probably still observable and not the effect of the different abutments on interleukin release.

Although, we did, indeed find a significant difference between the concentration of zirconia and the blood levels, there were more reasons to believe that it was a coincidence and that perhaps we were in the region of a 5% type 1 error rather than 95% certain.

There were no differences in IL6 levels at T0 between different abutments. The concentrations were all between 28 and 38 pg/ml a situation very similar to IL-1β levels, that were in the same range levels.

At T0 these results are in line with the theory/rationale that, at baseline, the interleukin pool available for extraction and analysis, is highly dependent on surgical trauma and the general health status of the sheep rather than biomaterial influence.

IL6 is an important mediator of fever and of the acute phase response and is responsible for stimulating acute phase protein synthesis, as well as the production of neutrophils in the bone marrow. It supports the growth of B cells and is antagonistic to regulatory T cells. (Banks, Kastin, and Gutierrez 1994)

It is also considered to be a myokine, a cytokine produced from muscle, which is heightened in response to muscle contraction. (Febbraio and Pedersen 2005)

The first incision line, in our study was made in the buccinatoris muscle, an anatomical area prone to IL6 induction and storage, which may explain the sudden rise of IL6 in all implants and abutments.

The comparison between IL6 and blood levels (BF) was interesting because, BF levels were taken after first incision, so they would, in theory, measure the amount of interleukin present in the sheep muscles and adjacent structures. However, the second measurement was 1 hour after the last stich was tied (T0), which in theory represented how fast the cell-to-cell communication can induce IL6 to the area and induce inflammation.

The results showed significantly less IL6 after 1 hour (T0) than at the incision time point (BF) (71,69 pg/ml).

In other words, there was significantly less IL6 expression 1 hour after incision time (BF) for each implant, independent of abutment biomaterial.

One can speculate that these values may be due to the available pool of native IL6 present at BF released by tissue trauma (IL6 at BF 76 pg/ml), but that later the reaction is no longer dependent on the amount of IL that was present but is mediated by plamocites, macrophages and other molecules which in theory are dependent on the strength of the sheep's immune system and its reaction time.

The depletion of IL6 levels due to surgery, saline washes and human manipulation, may explain why at T0 the IL6 concentrations levels dropped.

This later reaction has always been directly dependent on the immune system that triggered IL6 production. (76 pg/ml at BF and 38 ± 32, 28 ± 26, 37 ± 27 for Z, A and T respective at T0)

We observed in our study that the IL6 results (72 pg/ml) at the incision line (BF) are much higher that the values of IL-1β (16 pg/ml) at the same time frame.

These results alone prove that the concentration values found have a high probability of being correct, since these interleukin concentration results are in accordance with the human literature (Consolo et al. 2013). IL6 is a fast onset IL that is stored in the muscle tissues prepared for the acute phase and IL-1β is an immune modulated interleukin that only appears later in the chronic and established inflammation phase. This is why there are concentration discrepancies at the BF stage.

This control group is key to validating all the research, because if these findings had different values alone, it could affect the validity of all the other results.

Not only the results of IL-1β and IL6 at BF were in line with the literature but the IL-1β and IL6 concentrations in the periodontal sulcus also matched the human sulcular Interleukin concentration (Emecen-Huja et al. 2013). In this parameter, IL6 expressed higher concentrations than IL-1β in a healthy periodontium.

IL extraction at baseline was made 1 hour after surgery, which in theory is sufficient for macrophage stimulation of IL6, but we also conceded that the results could have been different if we had waited 2, 3 or 4 hours.

The consensus in the team was that the results would probably have showed higher and more homogenous concentrations of IL6.

The time frame of 1 hour following final stitiching, was decided because general anesthesia in sheep cannot be maintained indefinitely as the risk of sheep mortality was too high to risk another time frame.

Although baseline results showed that IL6 is present at that time, they were not found in higher concentrations than the periodontal crevicular tissues of the neighboring teeth, either at T0 or at T3.

These results were a surprise to us since in clinical visualization of the implant/abutment complex seemed to have a higher inflammatory status then the periodontal crevicular fluid (PCF) aspect of the sheep teeth.

This means that at T0 and at T3 the amount of IL6 expression is similar to the PCF expressed at both time frames (T0 and T3).

One of the possibilities for this clinical observation may be due to the measurement of only two inflammatory mediators. If there had been an

increase in the others it would not have been detected. Consequently, the more inflammation observed on implants than on teeth may very well be true, leading us to the question of whether or not we should have used more Interleukin mediators.

The clinical appearance of inflammation as red inflamed mucosa is mainly due to the overproliferation of blood vessels. The reaction of tissue to trauma or infection is to induce cell proliferation delivered to the area by increasing capillary networks.

There are a great number of Interleukins that can induce that state of angiogenic alterations, a phenomenon not exclusive to IL-1β or IL6. (Ericsson et al. 1995)

Another valid interpretation could be that the periodontal sulcus is also a constant area of interleukin production and so it would on average have the same interleukin concentrations, which seem unlikely, since all the sheep meals were controlled, and they were devoid of any component that would induce plaque or other inflammatory patterns in the sulcular area.

Only two inflammatory mediators (IL6 and IL-1β) were seen because these are the two main interleukins found in periodontal/peri-implant tissues that can stimulate bone resorption and loss.

In sheep, baseline parameters for both interleukins are not established in literature (and vague) so it is interesting to observe that the overall initial interleukin response in our study was similar to that expected in the human response (rfe). IL-1β and IL6 appear when they should, one in the acute phase and the other in the chronic phase respectively (although in a more residual form than the human response).

If the results had been divided by abutment and not by time frame a tendency towards similar IL6 behavior on all abutments (Z, A and T) would have been observed. There was a concentration drop from T0 to T1 and an increase in concentrations from T1 to T3. Although not statistically significant, this is an indication that implant placement is not an innocuous inert material for the sheep body.

Indeed, there was a continuous production of IL6 throughout the process of osseointegration. All the biomaterials behaved in the same way but the acrylic showed a higher tendency to express more IL6 in all stages of the measurements (T0, T1 and T3)

If one argued that at baseline the concentration levels were due to surgical trauma and not biomaterial influence, at T3 without any surgical intervention the oral tissues were still in an inflammatory state measured though IL6 and IL-1β production.

Thus, it is clear that implant placement induces a state of chronic peri-implat sulcular inflammation that resembles the PCF of neighboring teeth.

IL-1β could be key to understanding early healing immune response since the only parameter that was different from IL6 was the fact that the zirconia abutment induced less expression at T0 than at BF, a situation not found for T and A.

The same rational for IL6 could be used for BF where IL-1β concentrations are very low due IL needing time to be produced. It is thus normal that the values are higher at T0 than at BF. However, zirconia didn't follow this pattern but actually lowered the concentration value. One could argue that the same surgical manipulation that depleted IL6 at T0 could affect IL-1β in the same way.

The fact is that this situation happened in Z but not in A and T, meant that the concentrations found in A and T were higher than Z.

Based on the fact that T0 produced almost uniform results despite the abutment placed, it is our considered opinion that the result found for the zirconia abutment group should be interpreted with caution, as interleukin concentrations on the abutments were very similar to blood concentrations at T0.

Although there were no significant differences in inflammation levels, the pattern observed was that there was more inflammation at T3 than at the baseline for both interleukins

If the study is divided into material over the time events we see that the IL-1β has two separate behaviors, one in the T and Z abutment, and one in the acrylic.

In the T, an Z the values of IL-1β rise from T0 to T1 but revert to concentrations similar at T0 at T3. In the Acrylic, we saw a drop in the concentrations between T0 and T1 and then a rise to T3 surpassing the initial values of T0.

Although there was no statistically significance, the changes in IL-1β levels show a tendency for Acrylic to express more over the 3-month period than titanium and zirconia.

Although there was no recorded statistical significance, there was also a tendency for the implant to create a state of inflammation.

The surprising element of this is, if we admit that at T0 we are reading interleukin releasing as a result of trauma and not as a result of biomaterial modulation, the inflammatory pattern at T3 is higher than at T0, representing a higher state of inflammation than the trauma created by placing implants.

So here lies the difficulty in interpretation of results and the main motivation of this Phd thesis for beginning with an animal trial and proceeding to include a clinical trial. The lack of differences found at different time frames of the three abutments in the animal model could be attributed to the fact that there was really no difference between them, but in order to state this conclusively we needed strength in our sample, which we do not have.

The problem with the animal study's ws sample size, due to understandable budget issues and protocols.

An increase in sample size in the animal model would have been economically impossible for this study.

Our clinical observation was that the implant sites showed a more visible inflammation than the periodontal status of teeth, but IL analysis failed to show this. In fact there were similar results at T0 and at T3, with no statistical significance. It was with great interest at that time that we embarked on the clinical trial to see what the human RCT would reveal.

One of the advantages of this animal study was that sheep were treated equally treated in terms of feeding habits and medication, providing acceptable systematic control of the results whereas the individual systemic status of each human subject may be source of confounding factors. The sheep results may be interpreted as being that there was no abutment impact on the inflammatory levels. However, the graphics clearly indicate a rise in the inflammatory levels at T3, that were not present at T0. Moreover, when we compared blood levels at T0, there was a tendency for IL PICF levels to be higher than the inflammatory levels of the blood sampled. But once again these results should be cautiously interpreted.

The sheep failed to express different inflammatory levels of IL-1β and IL6 at the time of implant placement. But we were not able to make that correlation in my opinion because of sample size.

In other words, a human trial was needed to answer this question.

The inflammatory pattern was very similar in the 3 biomaterials used and also from PICF when compared to PCF.

This experiment thus suggested not a withdrawal of clinical conclusions, but the added proof needed in preparing for the RCT study in human samples.

Several problems that surfaced in this animal study were corrected and the RCT human trial clearly profited from this.

The extraction protocol was optimized at the IST, duplicates and triplicates were run to be sure of the concentration levels, but more importantly it gave us interleukin concentration values at different time frames which allowed us to choose the appropriate interleukin test for the human trial. Interleukin sensitivity is crucial because if the concentrations arere high and, for example you had a lower concentration kit sample there is the chance of failure in the readings.

Another important aspect of the animal work was inter- and intra- observer agreement in the ELISA methodology since the operator was calibrated on those steps, making the human trial less prone to concentration errors due to investigator methods.

The results of Part 2 in the randomized clinical control will be discussed and compared to the current literature on the subject.

These results showed that in a controlled environment where individual systemic health is not a factor, there is no impact of the different biomaterials on the inflammatory parameters of the sheep.

It seemed to us that the differences in IL variations are too small to yield a sample size calculation based on these results.

SECTION 3.5 CONCLUSION AND CLINICAL IMPLICATIONS OF ANIMAL EXPERIMENTAL MODEL

With regard to expression of IL6 and IL-1β from T0 to T3, T, Z and A show similar behavior over time, expressing the same amount of IL, than the PIC of adjacent teeth, at similar time frames.

For IL6 at BL, T, Z and A express less IL, than the same IL present in blood.

Z abutments bring about significantly less expression of IL-1β and IL6 than the IL present in the blood at BL,

The weaker reaction triggered by Z abutments (measured in osteoclastic inducer IL-1β) but not by A or T, may be key to understanding if different materials give rise to different marginal bone remodeling patterns in the first days of healing.

CHAPTER 4. RESEARCH PROJECT PART 2 - RANDOMIZED CLINICAL TRIAL (RCT) STUDY

SECTION 4.1 MATERIALS AND METHODS

Task 1: Study Outline

A - Introduction: Previous Experience with inflammation Protocols

During the 2016 year out team ran an animal study as a preliminary study for the Randomized clinical control trial. The protocol was intended to produce a sample size calculation based on the different concentrations of interleukins found on the three-biomaterials used, but most importantly, to optimize interleukin extraction methodology on the day of surgery (baseline T0).

In the IST bioengineering unit (where cytokine samples were read), the sheep protocol was intended to show the range of interleukin IL6/ IL-1β concentrations that one could expect from the peri-implant sulcus.

The information provided allowed us to choose the correct ELISA test for the RCT based on the sheep interleukins concentrations.

The extraction methodology to extract interleukins from periopaper® to the ELISA wells, was also optimized in the sheep model.

Another important point was to decide at which moment the samples should be taken.

In the sheep model, there were no alterations between 1 month and 3 months, this was a drawback, but it was not possible from a cost point of vew to study extra points between this time frames. Based on this the decision was made that it would make the most economic sense only totake samples from day 0 (baseline) and at T2 (8 weeks) with no intermediate points of control.

The protocol was under the supervision PI Prof.Dr João Caramês and attempted to respond to the same PICO question and hypothesis as the animal study.

For the Animal Study Ethical Clearance was obtained from ORBEA and Protocol Clearance from INIAV-Direcção Geral de Alimentação e Veterinária/Instituto Nacional de Investigação Agraria e Veterinária.

For the RCT clearance was obtained from the Ethical Comittee of Lisbon University

The protocol was also approved by the Scientific Commission from the Faculdade de Medicina Dentária de Lisboa (FMDUL)

The previous study was a sheep model that followed a strict protocol of implant placement (the same implant as the proposed RCT) and abutment placement (CAD-Cam Zirconia, Acrylic and Titanium).

Inflammation harvest protocol was used which included placing sterilized periopaper® in the peri-implant sulcus for 20 seconds on the day of surgery (T0) and at 2 Months (T2). The harvested inflammation fluids were transported in dry ice to IST (Instituto Superior Técnico) where they were frozen to -80ºC until sample reading.

Five Types of inflammatory sample (for IL-1β and IL6) readings were made: 1-perimplant tissue fluid for CAD-CAM Zirconia Abutments 2-perimplant tissue fluid for CAD-CAM Titanium Abutments 3-perimplant tissue fluid for CAD-CAM Acrylic Abutments 4-Periodontal Inflammatory fluid from teeth (Control) 5- Blood samples on the day of surgery (Control).

The RCT focussed on the same fundamental principles and used the same experimental implant and implant abutments used in the animal study.

B - Human Subject review: Ethical Committee Protocol Clearance

Clearance was obtained from the Faculdade de Medicina Dentária de Lisboa Ethical Committee in 2015 which is referred to as: A Comissão de Ética para a Saúde da Faculdade de Medicina Dentária da Universidade de Lisboa (CES-FMDUL). (Appendix B)

C - Study Outline

The RCT will follow the outline shown in table 27 to 29.

Table 27 - RCT outline and Summary

		Recruitment Phase		
		Periapical and Panoramic X-Ray		
		Informed Consent - Inclusion Criteria		
		Randomization		
	Group Formation Zirconia Healing Abutments n=20	Group Formation Titanium Healing Abutments n=20	Group Formation Acrylic Healing Abutments n=20	
		Implant Placement		
		Measure Bone Loss, Healing abutment surface Characterization, Inflammatory levels		
To Investigator:				To Patients
Data Collection				Final Impression
Data Reading				Crown Placement
Publish Results				

Table 28 - Study Summary - Autoimmune host response Human Study (RCT)

Implant + Zr/Ti/ CAD-CAM Acrylic healing abutment placement	⇨	Nanometric Biomaterial degradation	⇨	Autoimmune/Inflammation Host reaction	⇨	Marginal Bone remodeling
Initiating Factor/Cause	⇨	Etiology	⇨	Effect	⇨	Clinical Repercussion
Intervention	⇨	AFM Material Analysis	⇨	Interleukin Measurement	⇨	Radiographic Measurement

Table 29 - Study Flow Chart - Clinical Days

Preparation	Recruitment	Inclusion Criteria	Informed Consent
	Panoramic/periapical		
Day 1 (T0-Baseline)	Implant Placement	Place Healing Abutment	Periapical/Fluid Measurement
Day 2 (T1 -8week)			Periapical/Fluid Measurement

Study outline description: Place dental Implants and randomized zirconia, titanium, acrylic or cad-cam acrylic abutments, torque to 20 n/cm^2. Evaluate changes in inflammatory levels from T0 (baseline) to T2 (8 weeks). In addition, evaluate secondary outcomes: marginal bone loss, gingival height levels, osseointegration, gender, age, time of surgery, anatomical position and implant stability

D - Study Registration as Randomized Clinical Control Trial

Clinical trials have been described as the gold standard in the evaluation of therapeutic and preventive health issues. The registration of clinical trials has been proposed to comply with ethical grounds for those who participated in the study and informed that the research would be used to contribute to the development of science, regardless of the results. According to the World Health Organization (WHO), RCT and clinical trials should be reported and recorded before being started. This clinical trial was registered at the following database:

Clinical Trials Registries, http://clinicaltrials.gov under the registered name Implantology Institute, Portugal and has been assigned the number **NCT01961635** for free consultation. (appendix C)

E - Study Design

This randomized clinical trial is reported according to the CONSORT model ® for parallel clinical trial randomized non-inferiority reports.

3 Arms participated (60 subjects - 20 in each arm) with a common surgical phase in which platform-switch dental Implants were placed subcrestally

followed by three different two-piece healing abutments (CAD-CAM zirconium oxide (Zr), CAD-CAM commercially pure titanium IV (Ti), polimetacrilate CAD-CAM processed (PMC)

F - Study Clinical Locations

Lisbon University, School of Dentistry

This was a Unicentric Study conducted in Lisbon University, School of dentistry in the post-graduation program. The Specialization Course in Oral Surgery and Implantology trains health professionals, enabling them to fully address oral rehabilitation needs in the population. The course program follows the rules outlined by most associations of specialties within the area of Implantology, in Europe and in North America, and complies with the Community Directive that regulating the Dental profession and its associated specialties (78/686/EEC directive council of July 25, 1978), particularly in the requirement for 3 years full time study. Students in these courses participated in this study.http://www.fmd.ulisboa.pt.

G - Study Sample Reading Location

Instituto Superior Técnico (IST) - University of Engineering

The team worked in associationg with the the Institute for Bioengineering and Biosciences (IBB), a research unit at the Instituto Superior Técnico (IST), Universidade de Lisboa (UL), who are involved in cutting edge research and strategic advanced education in fundamental and applied biological sciences, biotechnology and bioengineering. The group is dedicated to responding to the challenge of exploring innovative approaches to key scientific and technological questions in biosciences and bioengineering and the transformation of scientific knowledge into tangible innovation.

The Institute was created in 2013, by the integration of the Bioengineering Research Group (BERG) and the Biological Sciences Research Group (BSRG), two research groups established at IST in 1991.

Our team, once again, worked directly with Prof. Gabriel Monteiro and his team in the biochemistry department.

Dra Sofia Duarte, a PhD Student in genetics and biochemistry, worked with us ensuring that the protocols were strictly followed and the readings accurately taken. The team was the same as for the animal model.

H - RCT Patient Inclusion criteria

Placement of single implants in any area (maxillary and mandibular) of extracted teeth for at least 3 months before implant placement, with sufficient bone volumes (at least 2 mm mesial, distal, buccal and palatal) to accommodate dental implants without the need for bone or soft tissue regeneration. Controlled oral hygiene, absence of any lesions in the oral cavity and at least 2 mm keratinized tissue. In addition, patients had to agree to participate in a postoperative control program and sign an informed consent.

I - RCT Patient Exclusion criteria

Patients were excluded from the study with the following conditions:

1 - Allergic to local anaesthetic, or any of the other components.

2 - Patients with hepatic or renal dysfunction

3 - Patients with epilepsy, shock, cardiovascular disorders or myasthenia gravis

4 - Patients with myocardial injury

5 - Hyperthyroidism

6 - Severe Hypertension

7 - Insufficient bone volume

8 - Smoking more than five cigarettes / day

9 - Excessive alcohol consumption

10 - Localized anti-tumour radiation therapy of the oral cavity

11 - Chemotherapy

12 - Liver Diseases

13 - Immunosuppressed patients

14 - Patients taking corticosteroids

15 - Pregnancy

16 - Inflammatory and autoimmune diseases of the oral cavity

J - Patient Organization - Baseline Formation

Initial data was collected for all patients selected for the trial, containing the following records: Biometric data, Clinical data collection, photographic data.

K - Recruitment

Recruitment was undertaken at School of Dentistry University Clinic (Lisbon), the trial opened to all and screening comleted. If the patient fell within the inclusion criteria they were eligible for the study.

L - Control and monitoring patient adhesion

A mechanism which included the front desk to make telephone calls and sms text messages reminding patients to keep their appointments.

M - Informed Consent

The study followed the regulations of Decree-Law n. º 309/2003, of 10 December, establishing a Health Regulatory Authority (LRA) statute, that obliges regulation and supervision of the activities and operation of establishments, institutions and services providers of health care. Informed consent was given by each patient. (appendix B)

N - Randomization Process

Randomization process was achieved according to the internet site:

Urbaniak, G. C., & Plous, S. (2011). Research Randomizer (Version 3.0) [Computer software]. Retrieved on April 22, 2011, from http://www.randomizer.org/

The process was a simple randomization without stratification groups for a similar allocation of secondary factors. It was executed through a process that involved no knowledge of the trial by clinicians, examiners or investigators generated from an algorithm created by computer, which created 3 sets of 20 numbers. Randomization was performed by an employee working at the University of Lisbon with the code (S2) and delivered in a sealed envelope to the principal investigator (PI).

The subjects were allocated a number from 1 to 60 consecutively in order of acceptance of the treatment plan and inclusion in the study. From this, the patient was placed in one group (intervention or control) according to previously generated randomization.

A co-investigator (I2) monitored the numbering process did not take part in the randomization assignment of the same number to different patients. I2 was responsible for coding the patient so that the principal investigator only had access to a number without knowing anything about the patient.

This information was stored on the personal computer of I2, a backup was made on CD (sealed) and delivered to the principal investigator and remained unoppened.

The clinician responsible for placing the healing abutment on the patient was informed on the day of surgery to perform the technique as follows:

The patient was identified by name in the reception (S2) the information was delivered to I2 via telephone, mail or in person (if present). The I2 informed the PI.

The PI provided the information, informed the S2 who informed the clinician and the clinical procedure was performed.

O - Blinding

The randomization process was blinded as previously described. Clinicians, investigators (except PI), and biostatisticians were blinded.

The person reading the data was also blinded. Surgeons who placed the implants were not blinded to the healing abutments but had no knowledge of the nature of the study.

The person who treated the data (the Biostatistician) was blinded.

P - Sample size calculation

Based on our animal study, there were no statistical differences in the inflammatory processes at T0 and T3. The differences were so minor that the sample size would have been probitively costly for any difference to be seen.

Having said this, and looking at the literature, sample size can be caluculated from the marginal bone loss described.

In one of the most representative studies on bone tissue behaviour and biomaterials,

"Loaded custom-made zirconia and titanium implants show similar Osseo integration: an animal experiment. Kohal RJ, Weng D, BachleM, StrubJR. J Periodontol 2004; 75:1262-1268"

The author studied the behaviour of titanium and zirconia implants and aspects of osseointegration.

An averge mineralized bone-to-implant contact after 9 months of healing and 5 months of loading amounted to 72.9% (SD: 14%) for the titanium implants and to 67.4% (SD: 17%) for zirconia.

To detect a difference between the 3 types of abutments in this trial, marginal bone loss of 2 mm (the amount considered to be clinically significant) and rejecting the null hypothesis with a significance level of two-sided (1-Alpha) of 95% and a power (1-beta, % chance to detect an effect) of 80% is needed. In addition, there is a need to maintain a ratio between the exposed and the unexposed group equal to 1. This includes, in a sample of 88 abutments (22 on

each arm of the study), those that are exposed (3 study arms - zirconia oxide, acrylic CAD-CAM) and not exposed (1 arm study - titanium).

This value was calculated in the following reference for calculation of sample:

Kelsey et al. Methods in Observational Epidemiology 2nd Edition, Table 12-15 Fleiss, Statistical Methods for Rates and Proportions, 3:18 & 3:19 formulas.

We calculated a drop out ratio-about 10%, and add 8,8 (8 rounded) more individuals in each arm of the study.

Q - Patient appointment control ("Flow Chart")

This was delivered at the point the patient entered the study, with a record of the clinical times which it is compulsory to attend, working as a calendar and as a way to control and monitor membership.

R - Drop out ratio and patient control

Patients who quit halfway through the treatment were given a "re-call" and the reason for quitting recorded in the case file.

Reasons for withdrawal will be shown in the final report.

S - Software

Patient information was inputted onto an SPSS (IBM Corp. Released 2011 IBM SPSS Statistics for Windows, Version 20.0, Armonk, NY: IBM Corp.) database programmed by the principal investigator.
No other person had access to this data.

T - Adverse effects related to Implantology

This clinical trial was based on procedures performed daily by dentists.

With regard to the use of medical devices we would not use anything that is not seen as reliable in the market as the objective of this study is to compare something that already exists.

However, any adverse effects were strictly monitored:

Implant failure below the normal osseointegration rate of 92 to 95%;

Bone loss due to infection or other causes that jeopardize local bone quality;

All investigators were alert to these items.

The study would have been discontinued had there been an abnormal number of implant failures. 10% or more was considered as the line past which the study would have been terminated.

U - Oral Hygiene flow chart and Patient control

Patients were schedule to make appointments with the oral hygienist 1 month prior to the surgical intervention and in a a regimen of every 6 months.

Task 2: Observer/investigator Calibration

A - Inter-observer agreement

To determine the degree of inter-observer agreement, a commonly used statistical tool known as the kappa coefficient (κ) was used. The following tests of agreement were done before the trial begans and, once again in the course of the clinical trial.

B - Inter-observer agreement in radiographic Marginal Bone Loss (MBL) reading

The reading of the clinical marginal bone loss was calibrated as follows:

a Kodak representative instructed clinicians in the calibration of X-rays with respect to the dimensions (and filters). Then we wereshowed three different radiographs and asked to evaluate the existing bone loss in mm. A degree of agreement of 80% was the amount considered ideal for moving forward with the study. This was recalibrated until this value was reached.

C - Inter-observer agreement - Clinical torque the abutment

Cinical preparation was performed using the company manual as a guide. Following this, three questions were asked regarding the prosthodontic procedure and only when the clinicians involved reached a 100% agreement, did the study begin.

D - Inter-observer agreement - Clinical protocol in implant preparation

The clinical preparation was calibrated using the Biomet-Zimmer ™ catalogue. Five questions were asked regarding implant placement, and the procedure only begun after the 3 clinicians involved had reached 100% agreement.

Task 3: Biomaterial Characterization and quality control

The Characterization of the experimental implant (Biomet-Zimmer T3 implant) and also the experimental healing abutments (Z, A and T) have already been discussed in Part one of this thesis**.**

A - Radiographic Characterization of the Implant-Abutment complex

FIGURE 89 - Clinical implant-abutment situation. Note the two-piece healing abutment in a platform switch implant.

FIGURE 90 - Radiography calibration of the microgap in the different implant abutment situations

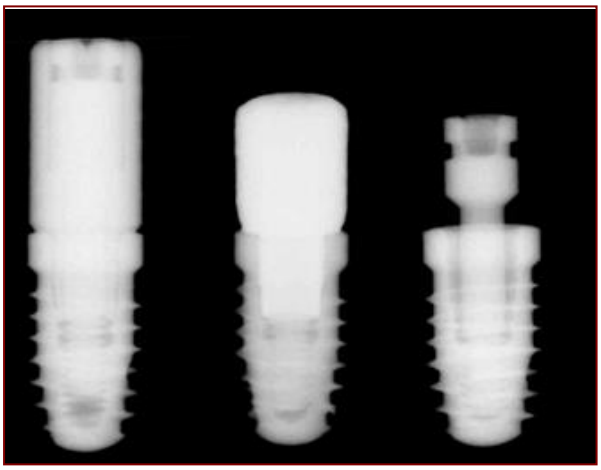


FIGURE 91 - Radiographic results of the implant abutment situation, calibrated in order to define how a standard procedure should be.

B - Description of the healing abutments sterilization procedure

The cad-cam zirconia and acrylic (milled) healing abutments underwent a process of autoclaving before placed in the patient's mouth on the day of surgery.

The T abutments were sterilized by the implant manufactures and did not go through this cycle. The following steps were undertaken in the sterilization procedure**.**

1. Placement of the material in heat sealable bags with colour change for chemical control

2. Placing the packaged material and performed sterilization cycle bagged stock (121-132 º) in the autoclave.

3. Once the program was completed, the bags were observed to see if they had changed colour. If there was no colour change, they were re-sterilized.

4. The material was put through the sterilization cycle successfully and stored until needed.

Task 4: RCT Intervention Phase

A - Formation of 3 Groups (3 Arms)

Control group (not exposed) (GC) CAD-CAM Two-piece healing abutment-Titanium (Place Titanium one-time one-abutment in subcrestal platform-switch dental implants on the day of implant installation, torque 20 n/cm2)

Experimental Group (Exposed) (GE) CAD-CAM two-piece healing abutment-Zirconia Oxide, CAD-CAM Acrylic (Place zirconia/CAD-CAM Acrylic one-time one-abutment in subcrestal platform-switch dental implants on the day of implant installation, torque 20 n/cm2)

B - Description of Surgical/prosthodontic procedure (intervention equal to all groups)

B1 - Anaesthesia

The area where the needle was going to be inserted was treated by means of topical anaesthesia lidocaine hydrochloride 2% (Laboratories Ltd. Volta Santiago, Spain), followed by a period of waiting of two minutes (recommended by manufacturer) before giving the injection. Arthicaine cloridrate 4% and epinephrine 1:100.000 (Laboratórios Inibsa, Barcelona/Espanha) were administered by means of a carpull and subperiosteally in the buccal and lingual. The latency time was 130 seconds before commencing any surgical procedure.

B2 - Surgical Technique measuring crest dimensions

Prior to any surgical procedure the amount of residual gingival tissue (residual biological width) was measured with a calibrated endodontic file (size 80).

The value obtained was measured by ruler. This procedure was intended to check the residual biological width in the future implant area.

This height was exposed to inflammatory protocols and marginal bone resorption.

B3 - Surgical Technique

Mid-crestal incision was undertaken with scalpel blade 15c, opening up a full-thickness mucoperiosteal flap with periosteum retractors for access to the basal bone.

B4 - First Surgical Phase-Implant Placement

All Patients underwent to a strict oral hygiene protocol: 2 weeks before implant surgery and one week after crown placement.

The experimental implant used was the Biomet-Zimmer ® Platform Switch T3 implnat. Implant microgeometry surface preparation presented a textured surface ((sandblasted with resorbable particles (sand blasted), with 75µ (large grit) titanium spheres and bathed in a solution of nitric acid (acid etch)) using the Biomet 3I® protocol for T3 platform-switch Implant (Recommended by Manufacturer). Below is the step-by-step implant placement protocol.

B5 - 1st Stage Surgery: Implant Placement

Implant Placement Protocol Tapered Biomet 3i ® (Indicated by Manufacturer)

Step 1 - Initial Preparation

The alveolar ridge was reduced and smoothed (if necessary) with a round bur to obtain a sufficiently large flat bone surface.

Step 2 - Implant Bed Marking

The site preparation set was marked for purpose during the planning, using a drill spear (ACT Pointed starter drill).

Step 3 - implant axis marking for Subcrestal placement.

The implant site was marked with the pilot drill Ø 2 mm, prepared to a depth of approximately 6 mm. The short edge of the depth gauge was inserted to verify the proper orientation of the implant axis.

Step 4 - Preparation of the implant site for the 2 mm

With the Ø 2 mm pilot was drilled to reach final depth. An alignment pin was used to check the axis of the implant and depth of preparation.

Step 5 - Extension of the implant site with quad shaping drills Ø 3.25 mm

For larger diameter implants two more protocol drills (4 mm for placing a 4-mm diameter implant) were used.

Step 6 - Extension of the implant site with quad shaping drill Ø 4 mm

Step 7 - Extending the implant site with bone drills dense tap Ø 4 mm (if necessary)

Step 8 - Subcrestal implant placement.

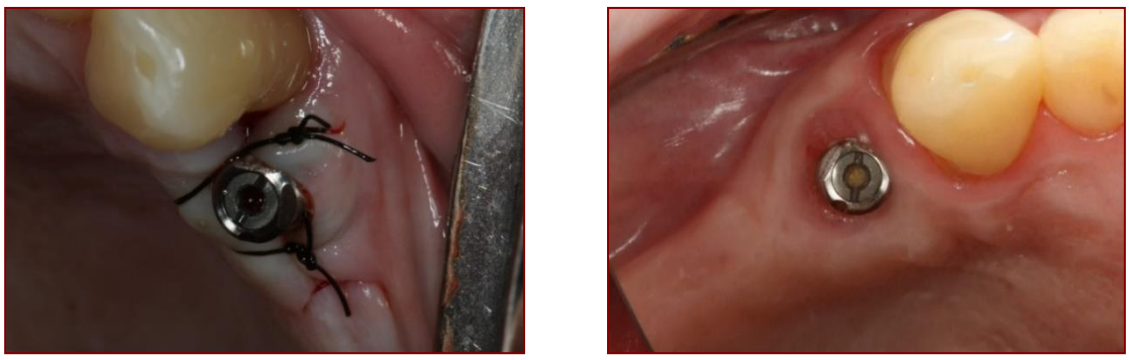


FIGURE 92 - Initial T0 baseline situation (left side) and the final situation at T2 with the titanium healing abutment complex**.**

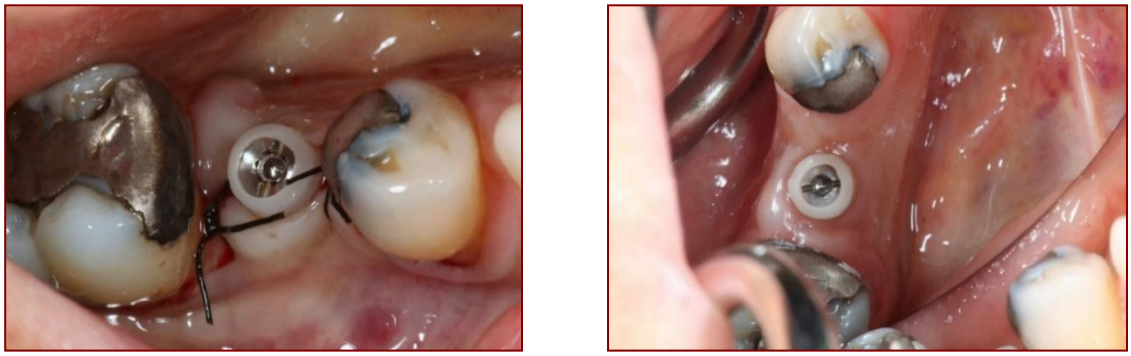


FIGURE 93 - Initial T0 baseline situation on the left and the final situation at T2 with the Acrylic healing abutment complex on the right.

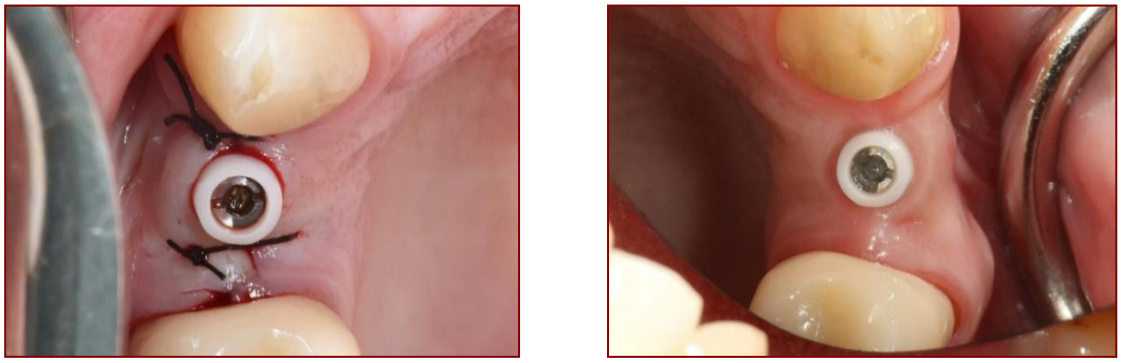


FIGURE 94 - Initial T0 baseline situation (left side) and the final situation at T2 with the Zirconia healing abutment complex

B6 - Technique and types of suture

Monofilament coated polyvinyl length 40 mm Coated PolivinilSuture (Sweden and Martina®, Italy) was used to suture. It is a sterile non-resorbable synthetic material comprising a polyvinyl copolymer.

The suture was non-absorbable and thust completely inert in terms of inflammation protocols.

B7 - Description of medication protocol Intra / pre-and post-operative

Two grams of Amoxicillin (Cipamox 1000 g, Atral Laboratories SA Santarem / Portugal) were administered orally one hour before. An analgesic was prescribed (1 g paracetamol - Ben-u-ron 1000g (bene-Arzneimittel GmbH, Munich / Germany)) for use in SOS postoperatively.

Patients were instructed to use mouthwash with chlorhexidine 0.2% (Corsodyl, GlaxoSmithKline) 3 days before, 2 times daily for one minute and for 3 more days after surgery.

B8 - Prosthodontic flow chart (not part of the study)

After final cytokine samples were taken, the patient officially ended his/her participation in the study. He/she was directed to the prosthodontic department to rehabilitate the implant**.**

The final prosthodontic procedure included Final impression - Intermaxillary record - Colour Registration - Final crown.

C - Marginal Bone Loss Assessment

Marginal bone loss is one of the primary outcome study items.

A parallel periapical standardized radiograph was set to measure bone position in relation to the implant platform at exactly the position intended at T0 and at T2.

An independent assessor performed intraoral radiographs by means of a personalized support (Rinn-XCP XCp-ds® digital sensor holders Dentsply® Germany) and a parallel technique.

The parallel technique used in this trial was based on the articles of Cunha in 2013, Rocha 2016 and Moergel 2016. (Cunha et al. 2013; Rocha et al. 2016; Moergel et al. 2016).

The technique consisted of a silicone impression material (putty) index of the patient bite register in the Rinn XCP support, and another silicone impression material (putty) index on the rim that hdld the collimation tube, as shown in fig.95.

The individualized support was marked with the patient personal study number and stored until T2 for final periapical x-ray.

The support holds a radiographic film that is read in the Vista Scan Mini View image plate scanner from Durr Dental ® (DÜRR DENTAL SE Bietigheim-Bissingen, Sweden)

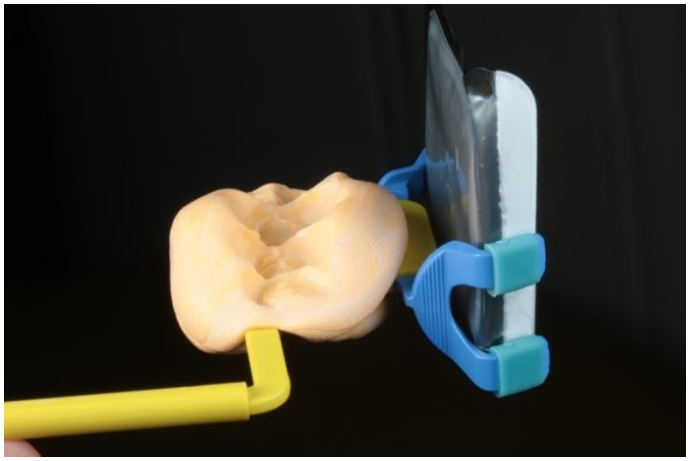


FIGURE 95 - Parallel intraoral radiograph, with a putty index for correct position of the film at T0 and T2, allowing implants to be consistently radiographed in the same position.

FIGURE 96 - Phosphorous dental film positioned in the Rinn XCP support.

FIGURE 97 - The putty index for the collimated tube, allowing for the consistent control of the x-ray beam in the same position.

FIGURE 98 - The putty index for the collimated tube, allowing for the consistent control of the x-ray beam in the same position.

FIGURE 99 - Intraoral x-ray support with putty index.

FIGURE 100 - The parallel technique. Note the parallelism of the x-ray support and the horizontal plane.

FIGURE 101 - The parallel technique. Note the parallelism of the x-ray support and the horizontal plane.

All radiographs were displayed and read on an image analysis program (Kodak Digital Imaging Software 6.11.7.0, Eastman Kodak, Rochester, NY, USA) on a 24-inch screen. LCD (Liquid Crystal Display) (iMac, Apple, Cupertino, CA, USA) and evaluated under standardized conditions (ISO 12646:2004).

The software was calibrated for each image using the known distance between the implant diameter and the length. The distance between the implant platform and the upper crest of the bone area was designated as bone loss.

The first step for marginal bone loss reading is to calibrate the image.

The known measurements in the x-ray are multiple, but the mesial-distal platform length (Biomet-Zimmer® is 4.1) was chosen. Firstly, the platform length was measured and assigned the value of 4,1 mm which is the size of the experimental implant.

Marginal bone loss was measured after the software was calibrated, as described in fig. 102 and more in detail on chapter 2.5.

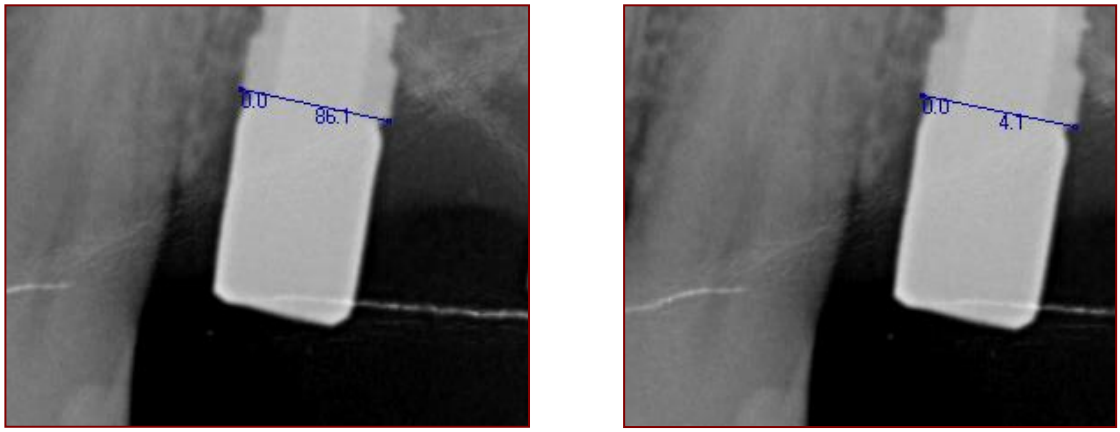


FIGURE 102 - Step by step marginal bone loss measurements. The first step is to calibrate implant platform.

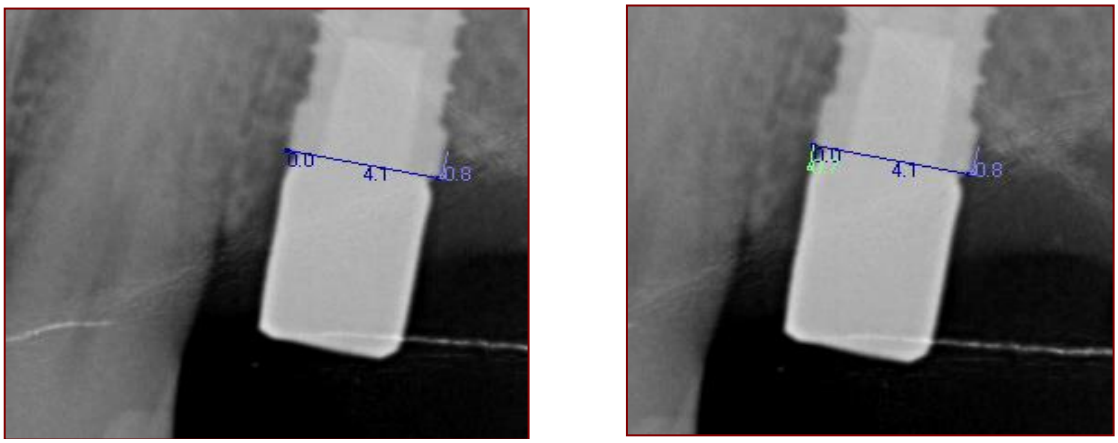


FIGURE 103 - Step by step marginal bone loss measurements. The second step is to measure mesial and distal bone loss with the calibrated scanner.

D - Primary Stability - Osseointegration ISQ measures

ISQ, or Implant Stability Quotient, is a scale from 1 to 100 and is a measure of the stability of an implant. The ISQ scale has a non-linear correlation to micro mobility. We now know that high stability means >70 ISQ, between 60-69 is medium stability and < 60 ISQ is considered as low stability.

A sensor is mounted on top of the implant and is then vibrated by gently moving it with magnetic pulses. The sensor will vibrate for a short while and then stop. If the implant stability (stiffness of the bone-implant interface) increases, the vibration frequency of the sensor will increase.

3 stability readings y of the implant mesial, distal and top were taken.

Stability readings were taken at implant placement T0 and at T2 (8 weeks).

E - Time of surgery Evaluation

The duration of surgery was measured from the first incision until the last suture.

F - Extraction of Blood Samples

The protocol was formulated from our experience with the sheep model.

Firstly, the edentulous area was rinsed with air and water and isolated with cotton rolls. After mid-crestal incision, a periopaper® strip was placed in the center of the incision until completely permeated (in 1 or 2 seconds)

Four strips (4) were placed in an Eppendorf tube and stored in dry ice for transportation.

G - Periodontal Cytokines extraction method

FIGURE 104 - Periodontal (PCF) extraction of Cytokines with periopaper in a protocol similar to the previous animal study in sheep.

The protocol was undertaken with the experience of the sheep model done and also from the works of Huja 2015. (Emecen-Huja, Hasan, and Miller 2015)

Firstly, the tooth was rinsed with air and water, and isolated with cotton rolls, and a periopaper® tip placed in the periodontal sulcus for 20 seconds in order to discharge the initial exudate.

After 20 seconds a Periopaper ® tip was inserted 1 mm inside the sulcus until a

slight resistance was felt for 20 seconds. Four strips (4) were placed in an eppendorf tube and stored in dry ice for transportation

H - Peri-implant Cytokine Extraction of IL-1β and IL6 at T0 (baseline) and at T2 (8 weeks)

FIGURE 105 - Zirconia healing abutments at T2.

FIGURE 106 - Peri-implant crevicular fluid (PICF) extraction of Cytokines with periopaper® in a protocol similar of the previous animal study in sheep. (note the adsortion on the tip)

The protocol was followed according to our experience in the sheep model and also from the works of Emecen-Huja. (Emecen-Huja, Hasan, and Miller 2015)

Firstly the abutment was rinsed with air and water, and isolated with cotton rolls and a periopaper® tip placed in the implant sulcus for 20 second to discharge the initial exudate.

After 20 seconds a periopaper® tip 1 mm was inserted inside the sulcus until a slight resistance was felt for 20 seconds. Four strips (4) were placed in an Eppendorf tube and stored in dry ice for transportation.

At T0 samples were drawn 30 minutes after the last suture.

I - Post-op Instructions

The patient received oral and written post-op instructions for correct cleaning maintenance protocols.

J - Post-op/Follow-up

After T0 the patient was instructed to come to the clinic after 8 days for suture removal and plaque control.

Task 5: Interleukin Sample handling and process

A - Specimens Treatment from surgery to storage

All Periopaper™ cytokines samples were stored in Eppendorf tubes and were taken from clinical harvest location (University of Lisbon, School of dentistry) to sample storage location, embedded in dry ice and immediately kept at -80 degrees at IST (Instituto Superior Técnico) for all the experiment work. All reagents (ELISA kits) were also stored at IST in the -20 degrees freeze chamber in the Biochemistry Unit of the same institution.

B - Interleukin (IL6 and IL-1β) Extraction Method (common to periodontal/peri-implant/blood samples)

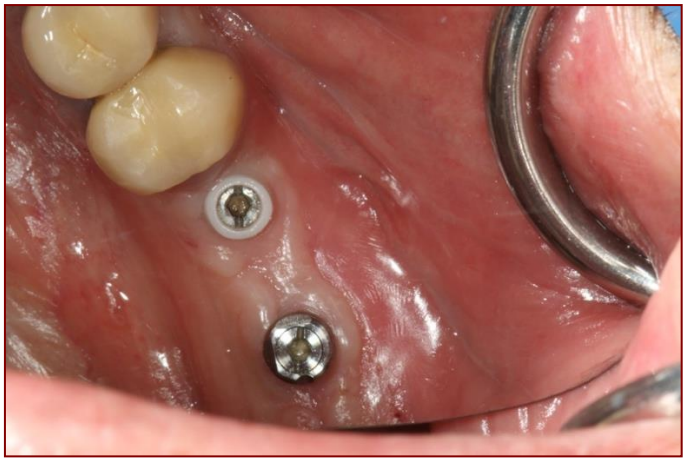


FIGURE 107 - Peri-implant healed region at T2 with Z and T healing abutments

The extraction method was performed, followed by the optimization procedure, as performed in the sheep model together with the ELISA manufacturer recommendations.

The reagents (ELISA kits) were brought to room temperature (18-25℃) before use, and once the experiment had been completed they were again stored in the -20ºC chamber to maintain the chemical properties.

The cytokines samples were taken from the -80℃ freezer before each sample test.

Sample (interleukins) preparation followed the aliquots procedure dilutions for calibration curve construction and the procedure was carried out according to manufacturer instructions.

C - Processing Peri-implant, Periodontal and Blood IL-1β, IL6 Samples

The interleukin extraction protocol from periopaper® adsorbent paper to Elisa wells, was similar for all three groups (the peri-implant samples, periodontal samples and blood samples).

First the periopaper® (adsorbent paper) was cut in two, with a sterilized scissor, leaving the wax part off, using only the adsorbent paper part for cytokines extraction.

The wax part was discarded, placing the white adsorbent paper part, in an Eppendorf tube with 200 ml of coated buffer solution (PBS) and left on ice for 30 mn. The Eppendorf tubes with a pocket-centrifuge every 10 mn (D1008E Mini Centrifuge Pocket Centrifuge (Centrifuge Rotor 5000rpm 1500g 110~220V™).

Following interleukin extraction at low temperature and with PBS washing buffer, the Eppendorf tubes were placed in a centrifuge (10 mn at 4º degrees Celsius with 12.000 RPM) for complete protein extraction. (Centrifuge Eppendorf 5810/5810 R™)

After centrifugation, the ELISA kit wells were filled, (calibrated pipette), with 100 ml (per well) for IL6 and 100ml for IL-1β reading.

FIGURE 108 - Cytokine extraction protocol. The Eppendorf tubes show aliquots and calibration curve samples before reading

FIGURE 109 - Ice storage for the 30 min buffer solution samples. Cytokines extraction methodology

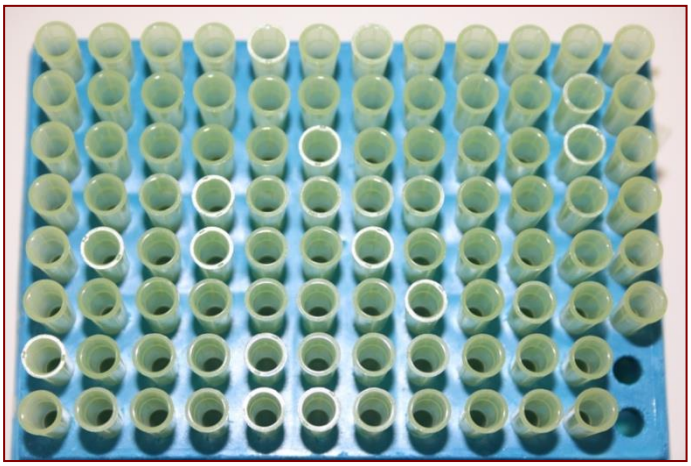


FIGURE 110 - Samples in the Eppendorf tubes ready for interleukin extraction protocol**.**

D - Assay Principle for IL6 and IL-1β

The Elisa kits were all validated kits for the purpose intended. They are from the commercial brand Booster-Bio ®.

The ELISA Kit was based on standard sandwich enzyme-linked immune-sorbent assay technology, a monoclonal antibody derived from a mouse specific for IL6/ IL-1β precoated onto 96-well plates. Standards (Expression system for standard: E. coli; Immunogen sequence: P29-M212) and test samples were added to the wells, a biotinylated detection polyclonal antibody from derived from a goat specific for IL6/IL-1β was subsequently added followed by washing with PBS buffer.

Avidin-Biotin-Peroxidase Complex was added, and unbound conjugates were washed away with PBS buffer. HRP substrate TMB was used to visualize HRP enzymatic reaction. TMB was catalysed by HRP to produce a blue colour product that changed into yellow after adding acidic stop solution. The density of yellow is proportional to the amount of Human IL6/ IL-1β in the sample captured on the plate.

Each ELISA plate is composed of 96 wells, and the wells can be read independently in rows of 8.

E - Interleukin 6 (IL6) ELISA Preparation and Testing

F - IL6 Sample Dilution Guideline

The concentration of the target protein (Periodontal/peri-implant/blood) present in the sample needed to be estimated and an appropriate dilution factor selected in order for the diluted target protein (IL-1β /IL6) concentration to fall in the vicinity of the middle of the linear regime in the standard curve.

Based on our animal sheep study, a very low target protein concentration (0pg/ml-4,69pg/ml) was assumed, so no dilution was performed.

G - IL6 ELISA

Based on the sheep animal study, we knew that the concentrations expressed in pg/ml for both interleukins (IL6 and IL-1β) would be low. Bearing this inin mind, an Elisa kit with high affinity for IL proteins was chosen.

A proven PicoKine™ ELISA Kit for Human Interleukin IL6 from the Boosterbio ™ company was used.

Its detection rangew is 4,69pg/ml-300pg/ml, with a sensitivity of <0.3pg/ml and specificity for natural and recombinant Human IL6 with no detectable cross-reactivity with other relevant proteins.

The kit was ordered on-line, and shipped in wet ice and stored at 4℃. avoiding multiple freeze-thaw cycles.

H - ELISA Kit Validity and Inter/intra Assay Precision for IL6

Intra-Assay Precision (Precision within an assay) was assessed by means of three samples of known concentration tested on one plate.

Inter-Assay Precision (Precision between assays) of known concentrations was assessed by testing in separate assays.

The results are as follows.

Table 30 - IL6 Inter/Intra Assay precision for IL6

	Intra-Assay Precision			Inter-Assay Precision		
Sample	1	2	3	1	2	3
N	16	16	16	24	24	24
Mean(pg/ml)	16.3	98	179	18.2	99	185
Standard deviation	0.8	2.3	4.2	1.0	3.6	5.7
CV (%)	4.9	2.3	2.3	5.5	3.6	3.1

I - Reagent Preparation and Storage - Human Standard IL6

The Elisa results are expressed in optical densities (O.D) that have to be translated into a concentration, measured, in this experiment, in pg/ml. To pass from optical densities to concentrations we need to create a calibration curve, with the known concentrations reccommended by the manufacturer. To do that, the Human IL-6 standard provided in the ELISA kit had to be reconstituted.

Firstly, an IL-6 standard solution using one tube of IL-6 standard (10ng/tube) provided by the manufacturer was prepared.

For 10000 pg/ml of Human IL-6 standard solution, 1ml sample diluent buffer was added into one tube and kept at room temperature for 10 min while mixing thoroughly.

For 300 pg/ml of Human IL-6 standard solution, 0.03 ml of the above IL-6 standard solution was added into 0,97 ml sample diluent buffer and mixed thoroughly.

For 150 pg/ml→4,6875 pg/ml of Human IL-6 standard solutions, 6 Eppendorf tubes were labelled 150 pg/ml, 75 pg/ml, 37,5 pg/ml, 18,75 pg/ml, 9,375 pg/ml and 4,6875 pg/ml, respectively. 0,3ml of the sample diluent buffer aliquot was added into each tube. 0.3ml of the above 300 pg/ml IL-6 standard solution was added into the 1st tube and mixed. Again, 0,3 ml was transferred from the 1st tube to the 2nd tube and mixed and finally, 0,3 ml was transferred from the 2nd tube to the 3rd tube and mixed, and so on.

J - Preparation of biotinylated anti-Human IL-6 antibody working solution

The solution was prepared no more than 2 hours prior to the experiment as reccommendeded by the manufacturer.

The total volume prepared was based on the number of wells that we intended to test in each experiment. For example: 0.1 ml/well x (the number of wells) according to the formula, Initial concentration x Initial volume = Final Concentration x Final volume (Dilution Equation, Ci x Vi = Cf x Vf, where "C" and "V" represent concentration" (in pg per ml) and "volume" (in ml) and "i" and "f" represent "initial" and "final". (Allowing 0.1-0.2 ml more than total volume) Biotinylated anti-Human IL-6 antibody should be diluted in 1:100 with the antibody diluent buffer and mixed thoroughly. (i.e. Adding 1µl Biotinylated Anti-Human IL-6 antibody to 99 µl antibody diluent buffer.)

K - Preparation of Avidin-Biotin-Peroxidase Complex (ABC) working solution for IL6

The solution was prepared no more than 1 hour prior to the experiment as reccommended by the manufacturer.

The total volume was based on the number of wells that we intended to test in each experiment: 0.1ml/well x (the number of wells) according to the equation stated above. (Allowing 0.1-0.2 ml more than total volume)

Avidin-Biotin-Peroxidase Complex (ABC) was diluted in 1:100 with the ABC dilution buffer and mixed thoroughly. (i.e. Adding 1 µl ABC to 99 µl ABC diluent buffer.)

L - Preparation of PBS washing buffer for IL6

Washing buffer Preparation: Dissolve AR0030-E to 1000ml distilled water and adjust pH to 7.2~7.6. Finally, the total volume was adjusted to 1L.

M - Interleukin IL-1β ELISA Preparation and Testing

N - Sample Dilution Guideline For IL-1β

The concentration of the target protein IL-1β in the sample had to be estimated (Periodontal/peri-implant/blood) and a proper dilution factor selected so that the diluted target protein concentration fell near the middle of the linear regime in the standard curve. Based on the animal sheep study a very low target protein concentration (0pg/ml-3.9pg/ml) was assumed so no dilution was made.

O - For Interleukin IL-1β

Based on the animal study we knew that the concentrations expressed in pg/ml for both interleukins would be low. With this in mind we chose an Elisa kit with a high affinity for IL proteins.

For Interleukin, the Human IL-1β a proven kit, the PicoKine™ ELISA Kit from Boosterbio™ was used, with a detection range of 3.9pg/ml-250pg/ml, a sensitivity of <0,15pg/ml and a specificity for natural and recombinant Human IL-1β with no detectable cross-reactivity with other relevant proteins.

It was ordered online, shipped in wet ice and stored at 4 ℃, avoiding multiple freeze-thaw cycles.

P - Kit Validity and Inter/intra Assay Precision for IL-1β

Intra-Assay Precision (Precision within an assay) was assessed by testing three samples of known concentration on one plate.

Inter-Assay Precision (Precision between assays) was assessed by testing three samples of known concentration in separate assays.

The results are on the following table.

Table 31 - IL6 Inter/Intra Assay precision for IL-1β

	Intra-Assay Precision			Inter-Assay Precision		
Sample	1	2	3	1	2	3
N	16	16	16	24	24	24
Mean(pg/ml)	19	41.2	116	27.5	61.3	179.1
Standard deviation	1.0	2.6	6.5	1.6	4.1	12.7
CV(%)	5.3	6.3	5.6	5.8	6.7	7.1

Q - Reagent Preparation and Storage - Human Standard IL-1β

The Human IL-1β standard also had to be reconstituted by preparing IL-1β standard solution using one tube of IL-1β standard (10 ng/tube).

For 10000 pg/ml of Human IL-1β standard solution 1ml sample diluent buffer was added into one tube and the tube kept at room temperature for 10 min and mixed thoroughly.

For 250 pg/ml of Human IL-1β standard solution 0.25 ml of the above IL-1β standard solution was added into 0.75 ml sample diluent buffer and mixed thoroughly.

For 125 pg/ml→3.90625 pg/ml of Human IL-1β standard solutions: 6 Eppendorf tubes were labelled with 125 pg/ml, 62.5 pg/ml, 31.25 pg/ml, 15.625 pg/ml, 7.8125 pg/ml, 3.90625 pg/ml, respectively. Aliquot 0.3ml of the sample diluent buffer was introduced into each tube and 0.3 ml of the 250 pg/ml IL-1 Beta standard solution added into 1st tube and mixed. Then 0.3 ml. were transfered from the 1st tube to the 2nd tube and mixed, followed by transfer of 0.3 ml from the 2 nd tube to the 3rd tube and mixed, and so on.

R - Other Reagent Preparation

Preparation of biotinylated anti-Human IL-1β antibody working solution was prepared with Avidin-Biotin-Peroxidase Complex (ABC) working solution

and preparation of PBS washing buffer similar to IL6.

S - Calibration curve IL6

Standard Preparation for the Calibration Curve:

Standards were prepared within 15 minutes of use, with the centrifuge at 10,000 rpm for 1 minute (Centrifuge Eppendorf 5810/5810 R™), and the Standard reconstituted with 1.0 mL of Reference Standard and Sample Diluent, the lid tightened and left to stand for 10 minutes, turning it over several times. After it had fully dissolved it was mixed thoroughly with a pipette. This reconstitution produced a stock solution of 1000pg/mL for IL6.

The recommended concentrations of the ELISA plates according to the manufacturer were for IL6:

300 pg/ml, 150 pg/ml, 75 pg/ml, 37.5 pg/ml, 18.75 pg/ml, 9.375 pg/ml, 4.6875 pg/ml and for IL-1β: 125 pg/ml, 62.5 pg/ml, 31.25 pg/ml, 15.625 pg/ml, 7.8125 pg/ml, 3.90625 pg/ml

T - Calibration curve IL-1β

For IL-1β we decided to create an extra calibration point to increase specificity, the 3,9 pg/ml was diluted to 1,95 pg/ml, transforming the following concentration table from:

Table 32

Concentration(pg/ml)	0	3.9	7.8	15.6	31.2	62.5	125	250
O.D.	0.003	0.086	0.161	0.292	0.557	1.029	1.617	2.222

into:

Table 33

Concentration (pg/ml)	0	1,95	3.9	7.8	15.6	31.2	62.5	125	250
O.D.	0,003		0,086	0.161	0.292	0.557	1.029	1.617	2.222

In total, 31 wells were used to reconstitute the calibration curve in IL-1β

U - Calibration curve IL6

Table 34

Concentration(pg/ml)	0	4.69	9.38	18.75	37.5	75	150	300
O.D.	0.002	0.059	0.106	0.226	0.428	0.736	1.372	2.279

29 ELISA wells were used to reconstitute the IL6 Standard calibration curve

V - Assay procedure (common for both IL6 and IL-1β)

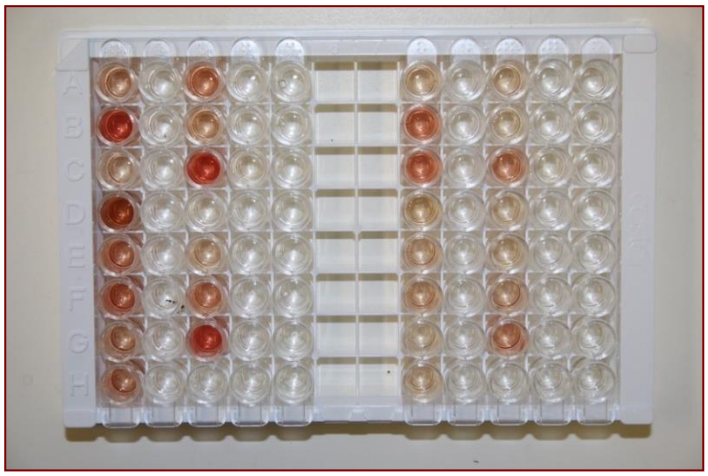


FIGURE 111 - Elisa plate sample distribution after extraction protocol, immediately before sample preparation for OD reading

The Elisa plate comprised peri-implant, periodontal and blood samples, but also with calibration curve points.

In order to do this 0.1 ml per well of the 300 pg/ml,150 pg/ml, 75 pg/ml, 37,5 pg/ml, 18,75 pg/ml, 9,375 pg/ml, 4,6875 pg/ml Human IL-6 standard solutions were aliquoted into the precoated 96-well plate, after which duplicates, triplicates and in some cases quadriplicates of the readings were taken to ensure accurate and reliable results.

For IL-1β, 0.1ml per well of the 250 pg/ml,125 pg/ml, 62.5 pg/ml, 31.25 pg/ml, 15,625 pg/ml, 7,8125 pg/ml, 3,90625 pg/ml Human IL-1β standard solutions were aliquoted into the precoated 96-well plate.

After the Elisa wells received the samples, the plate was sealed with a new adhesive cover provided and incubated at 37°C for 90 min.

After 90 mn the cover was removed, the contents of the plate discarded, the plate blotted onto paper towels. The wells were not completely dry at any time.

0.1ml of biotinylated anti-Human IL-6 antibody working solution was added into each well, the plate sealed with a new adhesive cover provided and incubated at 37°C for 60 min.

FIGURE 112 - ELISA plate after the biotinylated anti-Human has been place before the addition of the stop solution.

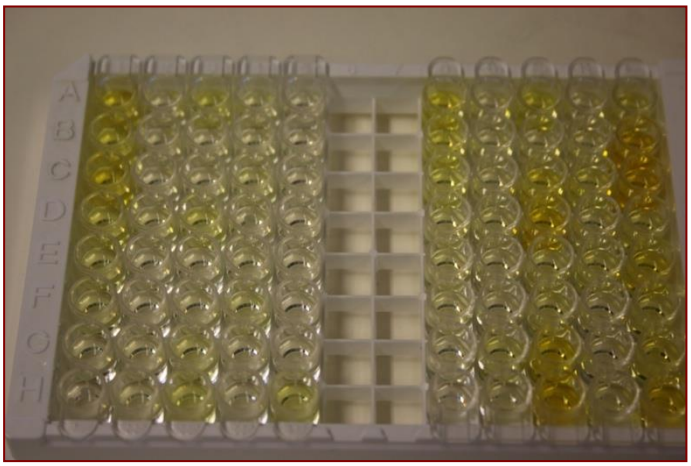


FIGURE 113 - ELISA wells before the addition of the stop solution.

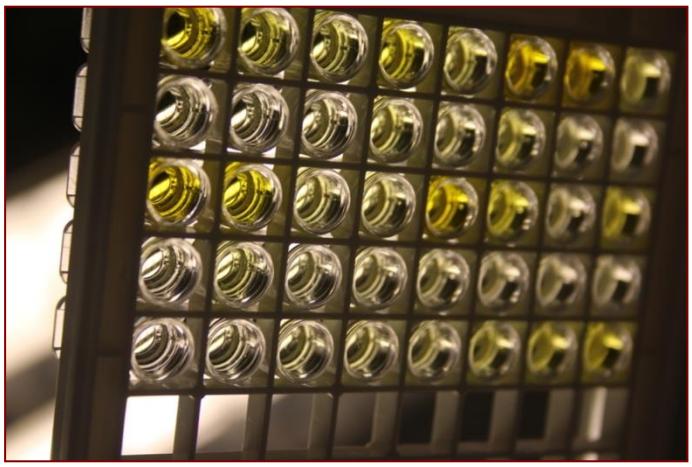


FIGURE 114 - Color intensity: the more intense the more inflammatory interleukins there are**.**

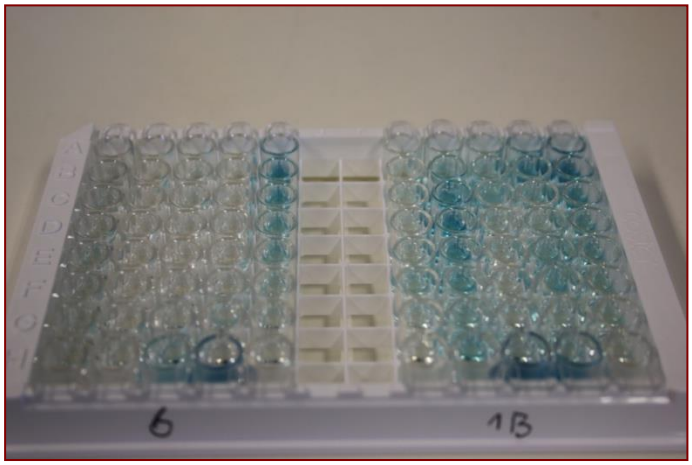


FIGURE 115 - Elisa plate immediately after addition of the stop solution and before OD reading.

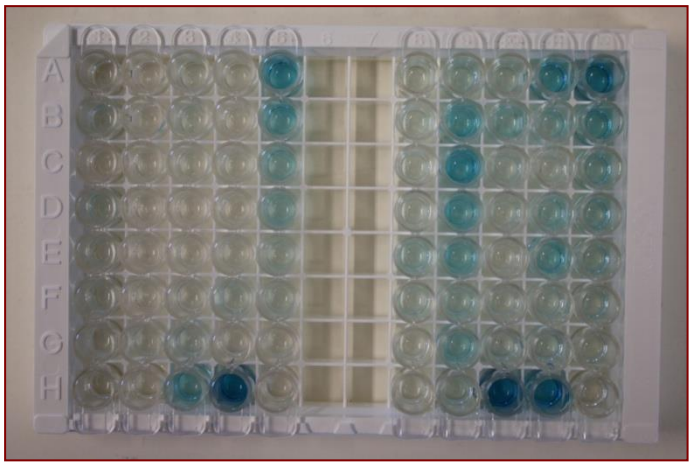


FIGURE 116 - Color intensity after addition of the stop solution.

After 60 min, the plate was washed 3 times with a multichannel pipette, with 0.01M PBS, and each time the washing buffer remained in the wells for 1 min.

after which the washing buffer was discarded and the plate blotted onto paper towels.

0.1ml of prepared ABC working solution was added into each well, the plate sealed with a new adhesive cover provided and incubated at 37°C for 30 min.

After 30 min, the ELISA plate was washed 5 times with 0.01M PBS, and each time the washing buffer was allowed to stay in the wells for 1-2 min. The washing buffer was discarded, and the plate blotted onto paper towels.

Following this, 90µl of prepared TMB colour developing agent was added into each well, l the plate sealed with a new adhesive cover and incubated at 37°C in the dark for 20-25 min.

0.1ml of prepared TMB stop solution was added into each well and the colour changed into yellow immediately.

Finally, the O.D. absorbance at 450nm was recorded on a microplate reader (SpectraMax Plus 384 Microplate Reader (Molecular Devices, USA) 5 min after adding the stop solution.

W - Sample Reading Well Distribution

At the beginning of the RCT we projected a total of 60 initial samples (T0) and 60 final samples (T1) making a total of 120 samples for ELISA reading.

After RCT completion the final numbers were altered, due to the loss of two implants in patient #48 and #56 respectively. The loss was osseointegration related, classified as early implant loss. In addition, patient #15, #18, #19, #22 dropped out the study due to change of address.

A total of 54 abutments was available for the final sample reading. From these 19 were acrylic, 18 zirconia and17 titanium, making two interleukins readings at T0 (baseline) and T2 (8 weeks) there were a total of 108 Elisa sample readings for Peri-implant Interleukin IL-1β and 6.

For Blood Interleukin 1β/6 there were 12 samples and for Periodontal IL-1β/IL6 there were 20 samples, making a total of 32 samples.

For IL-1β, 31 calibration points were used and 29 for IL6, making a total of 50 calibration points.

The ELISA plate 1 and ELISA plate 2 for Interleukin characterization (well distribution, considering 108 samples peri-implant samples, 54 initial and 54 final, 50 points of calibration curve with duplicates and triplicates for standard curve configuration, 20 Periodontal samples and 12 blood samples) are represented below.

Table 35 - ELISA IL-1β readings of Peri-implant/Periodontal/Blood and Calibration Curve Samples per well in the ELISA kit

500,00	62,50	15,60	3,90	#1T1	#5T1	#9T1	#13T1	#20T1	#25T1	#29T1
250,00	62,50	15,60	1,95	#2T0	#6T0	#10T0	#14T0	#21T0	#26T0	#30T0
250,00	31,20	7,80	1,95	#2T1	#6T1	#10T1	#14T1	#21T1	#26T1	#30T1
250,00	31,20	7,80	1,95	#3T0	#7T0	#11T0	#16T0	#23T0	#27T0	#31T0
125,00	31,20	7,80	0,00	#3T1	#7T1	#11T1	#16T1	#23T1	#27T1	#31T1
125,00	15,60	7,80	0,00	#4T0	#8T0	#12T0	#17T0	#24T0	#28T0	#32T0
125,00	15,60	3,90	0,00	#4T1	#8T1	#12T1	#17T1	#24T1	#28T1	#32T1
62,50	15,60	3,90	#1T0	#5T0	#9T0	#13T0	#20T0	#25T0	#29T0	#33T0

Table 36 - Plaque 2: ELISA IL-1β readings of Peri-implant/Periodontal/Blood and Calibration Curve Samples per well in the ELISA kit

#33T1	#37T1	#41T1	#45T1	#50T1	#54T1	#59T1	blood	Tooth	Tooth	Tooth
#34T0	#38T0	#42T0	#46T0	#51T0	#55T0	#60T0	blood	Tooth	Tooth	Tooth
#34T1	#38T1	#42T1	#46T1	#51T1	#55T1	#60T1	blood	Tooth	Tooth	Tooth
#35T0	#39T0	#43T0	#47T0	#52T0	#57T0	blood	blood	Tooth	Tooth	X
#35T1	#39T1	#43T1	#47T1	#52T1	#57T1	blood	blood	Tooth	Tooth	X
#36T0	#40T0	#44T0	#49T0	#53T0	#58T0	blood	blood	Tooth	Tooth	X
#36T1	#40T1	#44T1	#49T1	#53T1	#58T1	blood	blood	Tooth	Tooth	X
#37T0	#41T0	#45T0	#50T0	#54T0	#59T0	blood	Tooth	Tooth	Tooth	X

Table 37 - Plaque 1: ELISA IL6 reading of Peri-implant/Periodontal/Blood and Calibration Curve Samples per well in the ELISA kit

1000,00	75,00	18,75	4,69	#1T1	#5T1	#9T1	#13T1	#20T1	#25T1	#29T1
300,00	75,00	18,75	0,00	#2T0	#6T0	#10T0	#14T0	#21T0	#26T0	#30T0
300,00	75,00	18,75	0,00	#2T1	#6T1	#10T1	#14T1	#21T1	#26T1	#30T1
150,00	75,00	9,375	0,00	#3T0	#7T0	#11T0	#16T0	#23T0	#27T0	#31T0
150,00	37,50	9,375	0,00	#3T1	#7T1	#11T1	#16T1	#23T1	#27T1	#31T1
150,00	37,50	9,375	X	#4T0	#8T0	#12T0	#17T0	#24T0	#28T0	#32T0
75,00	37,50	4,69	X	#4T1	#8T1	#12T1	#17T1	#24T1	#28T1	#32T1
75,00	18,75	4,69	#1T0	#5T0	#9T0	#13T0	#20T0	#25T0	#29T0	#33T0

Table 38 - Plaque 2: ELISA IL6 reading of Peri-implant/Periodontal/Blood and Calibration Curve Samples per well in the ELISA kit

#33T1	#37T1	#41T1	#45T1	#50T1	#54T1	#59T1	blood	Tooth	Tooth	Tooth
#34T0	#38T0	#42T0	#46T0	#51T0	#55T0	#60T0	blood	Tooth	Tooth	Tooth
#34T1	#38T1	#42T1	#46T1	#51T1	#55T1	#60T1	blood	Tooth	Tooth	X
#35T0	#39T0	#43T0	#47T0	#52T0	#57T0	blood	blood	Tooth	Tooth	X
#35T1	#39T1	#43T1	#47T1	#52T1	#57T1	blood	blood	Tooth	Tooth	X
#36T0	#40T0	#44T0	#49T0	#53T0	#58T0	blood	blood	Tooth	Tooth	X
#36T1	#40T1	#44T1	#49T1	#53T1	#58T1	blood	blood	Tooth	Tooth	X
#37T0	#41T0	#45T0	#50T0	#54T0	#59T0	blood	Tooth	Tooth	Tooth	X

X - Software and Data analysis

Patients were registered on a database in SPSS (IBM Corp. IBM SPSS Statistics 2011 Released for Windows, Version 20.0 Armonk, NY: IBM Corp.) by the principal investigator. No other person had access to this data.

Y - Database

Patients were booked in as normal patients at the center where the trial was held.

Clinical records, other than the trial data, are open to all other clinics.

Z - Data Protection

Patient data was only available to the principal investigator who stored them in a secure location which was accessible by password (computer data) or key (in the case of copies of "back-up").

AA - Overall sample size

Sample size was calculated for equal groups of 20 abutments for each material (Z, A and T) making a total of 60 healing abutments for statistical readings.

2 implants were lost, one in abutment 48 (with a titanium healing abutment) and one in abutment 56 (with a zirconia healing abutment) due to osseointegration failure (early implant loss).

Two patients moved to another country during the trial, not completing the study at T2. One patient had 3 healing abutments (abutment number 18 corresponded to a zirconia healing abutment,19 corresponded to a zirconia healing abutment and 22 corresponded to an acrylic healing abutment) and another had one zirconia healing abutment (number 15).

Healing abutment 54 and 55 should have received a titanium healing abutment but due to a logistical error, there was none available, and so each receive a zirconia healing abutment instead.

Table 39 - Healing abutment distribution by tooth position and material

Implant #	Tooth #	Material
1	47	A
2	45	A
3	25	A
4	16	Z
5	36	T
6	25	T
7	37	T
8	37	A
9	46	A
10	17	T
11	24	A
12	22	A
13	36	Z
14	26	T
15	46	Z (dropped out at T2)
16	11	Z
17	25	T
18	36	Z (dropped out at T2)
19	15	Z (dropped out at T2)
20	37	T
21	46	Z
22	16	A (dropped out at T2)
23	26	T
24	14	Z
25	14	Z
26	24	T
27	25	A
28	15	T
29	36	Z
30	36	A
31	24	T
32	46	T
33	46	Z
34	16	Z
35	24	T

36	45	A
37	26	T
38	24	Z
39	16	A
40	25	Z
41	15	Z
42	23	Z
43	14	Z
44	14	A
45	36	A
46	25	Z
47	24	A
48	35	T (early implant loss)
49	45	T
50	15	A
51	44	T
52	24	A
53	25	T
54	26	Z*
55	25	Z*
56	46	Z (early implant loss)
57	15	A
58	35	A
59	12	Z
60	34	A

*Abutments should have received titanium healing abutments according to randomization process, but due to technical reasons received zirconia healing abutments

For final extraction data, the final numbers were: 18 zirconia healing abutments, 19 Acrylic healing abutments, 17 Titanium healing abutments.

Healing abutment 10 (titanium), 29 (zirconia), 17 (acrylic) were read, but in IL-1β concentration values reached the upper limit of the concentration chart, not giving an accurate value, so they were set aside for statistical reading and final results.

Thus, for total Interleukin reading the IL6 was 54 both at T0 and T2, but for IL-1β was 54 at T0 and 51 at T2.

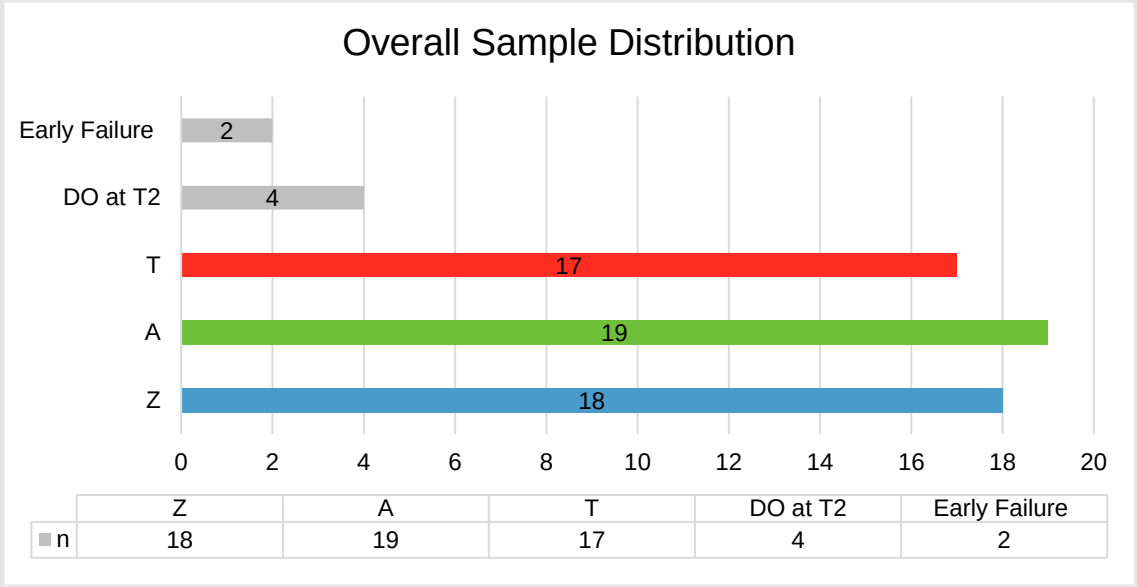


FIGURE 117 - Sample distribution. Final sample for Interleukin IL-1β and IL6 extraction. Two implants lost due to osseointegration failure, meaning two healing abutments were lost, 4 healing abutments DO (dropped-out) at T2 (8 weeks). Seventeen healing abutments for T (titanium), nineteen for A (acrylic) and eighteen for Z (zirconia)

AB - Statistical Methodology

The statistic methodology used was very similar to the animal model. To relate quantitative variables (such as IL6, IL-1β inflammation) to qualitative

variables (such as the material or moment, T0, T1 or T3), we proceed as follows:

When the qualitative variable had two cases (moment), the parametric test T was used, if the quantitative variable had a normal distribution or the samples in each of the two groups were large (more than 30). If any of these assumptions were not verified, then the non-parametric alternative Mann-Whitney test was used.

When there were 3 or more cases (material) in the qualitative variable, the parametric ANOVA test was used, if the quantitative variable had a normal distribution or the samples in each of the groups were large (more than 30), and there was homogeneity in the variances. If the assumptions were not verified, then the nonparametric alternative Kruskall-Wallis test was used.

For all tests in this study the significance level of 5% ($p \leq 0,05$) was considered

statistically significant.

AC - Results Inter-Observer agreement

Reproducibility of the examiners (AC, HF and JC) was assessed by calibrating in marginal bone loss reading, implant placement and torque.

A procedure for enhancing the verifiability of data involves comparing independent observations from two or more observers of the same events. IOA is calculated by taking the number of agreements between the independent observers and dividing by the total number of agreements and is added to the disagreements. The coefficient is then multiplied by 100 to calculate the percentage (%) of agreement.

All the examiners achieved 100% in the calibration test in the 3 methodologies.

CHAPTER 5. HYPOTHESIS AND RESULTS

SECTION 5.1. PRIMARY OUTCOME MEASURES: INFLAMMATION LEVELS OF IL6 AND IL-1B IN THREE DIFFERENT HEALING ABUTMENTS ZIRCONIA (Z) ACRYLIC (A) AND TITANIUM (T) - HYPOTHESIS AND RESULTS

Section 5.1.1. Hypothesis

Primary Outcome Measures: To relate the influence of abutment material on peri-implant inflammation in accordance with the following assumptions:

Correlation Between Inflammation Vs Abutment Material (Z, T, A)

Specific aim 1: For Overall Interleukins (IL-1β+IL6) Volume at Implant Placement T0 (Baseline)

H0: There is no difference in the total production of inflammatory reactions at T0 **(Interleukins IL-1β+IL6),** on titanium healing abutments, compared to zirconia or acrylic in implants placed under the standard protocol.

H1: There is a difference in the total production of inflammatory reactions at T0 **(Interleukins IL-1β+IL6),** of titanium healing abutments compared to zirconia or acrylic in implants placed under the standard protocol.

Specific aim 2: For Overall Interleukins (IL-1β+IL6) Volume at T2 (8Weeks)

H0: There is no difference in the total production of inflammatory reactions at T2 **(Interleukins IL-1β+IL6)**, on the titanium healing abutments compared to zirconia or acrylic in implants placed under the standard protocol.

H1: There is a difference in the total production of inflammatory reactions at T2 **(Interleukins IL-1β+IL6**), of titanium healing abutments compared to zirconia or acrylic in implants placed under the standard protocol.

Specific aim 3: For Overall Interleukins (IL-1β+IL6) Volume from T0 to T2

H0: There is no difference in the total production of inflammatory reactions from T0 to T2 **(Interleukins IL-1β+IL6)**, of titanium healing abutments compared to zirconia or acrylic in implants placed under the standard protocol.

H1: There is a difference in the total production of inflammatory reactions from T0 to T2 **(Interleukins IL-1β+IL6)**, of titanium healing abutment compared to zirconia or acrylic, in implants placed under the standard protocol standard.

Specific aim 4: For Interleukin (IL6) At Implant Placement T0 (Baseline)

H0: There is no difference in the total production of inflammatory reactions at T0 (**Interleukin IL6**) of titanium healing abutments compared to zirconia or acrylic in implants placed under the standard protocol.

H1: There is a difference in the total production of inflammatory reactions at T0 (**Interleukin IL6**), on titanium healing abutments compared to zirconia or acrylic in implants placed under the standard protocol.

Specific aim 5: For Interleukin (IL6) At T2 (8Weeks)

H0: There is no difference in the total production of inflammatory reactions at T2 (**Interleukin IL6**) of titanium healing abutments compared to zirconia or acrylic in implants placed under the standard protocol.

H1: There is a difference in the total production of inflammatory reactions at T2 (**Interleukin IL6**) of titanium healing abutments compared to zirconia or acrylic in implants placed under the standard protocol.

Specific aim 6: For Interleukin (IL6) From T0 to T2

H0: There is no difference in the total production of inflammatory reactions from T0 to T2 (**Interleukin IL6**) on titanium healing abutments compared to zirconia or acrylic in implants placed under the standard protocol.

H1: There is a difference in the total production of inflammatory reactions from T0 to T2 (**Interleukin IL6**) of titanium healing abutments compared to zirconia or acrylic in implants placed under the standard protocol.

Specific aim 7: For Interleukin IL-1β At Implant Placement T0 (Baseline):

H0: There is no difference in the total production of inflammatory reactions at T0 (**Interleukin IL-1β**), on titanium healing abutments compared to zirconia or acrylic, in implants placed under the standard protocol.

H1: There is a difference in the total production of inflammatory reactions at T0 (**Interleukin IL-1β**), of titanium healing abutment compared to zirconia or acrylic, in implants placed under the standard protocol.

Specific aim 8: For Interleukin IL-1β At T2 (8Weeks)

H0: There is no difference in the total production of inflammatory reactions at T2 (**Interleukin IL-1β**), on titanium healing abutments compared to zirconia or acrylic in implants placed under the standard protocol.

H1: There is a difference in the total production of inflammatory reactions at T2 (**Interleukin IL-1β**), on titanium healing abutments compared to zirconia or acrylic in implants placed under the standard protocol.

Specific aim 9: For Interleukin IL-1β From T0 to T2

H0: There is no difference in the total production of inflammatory reactions from T0 to T2 (**Interleukin IL-1β**), on titanium healing abutments compared to zirconia or acrylic in implants placed under the standard protocol.

H1: There is a difference in the total production of inflammatory reactions from T0 to T2 (**Interleukin IL-1β**), on titanium healing abutments compared to zirconia or acrylic in implants placed under the standard protocol.

Section 5.1.2. Results

IL-1β and IL6 Elisa Plate OD Readings (Titanium (T), Zirconia (Z), Acrylic (A), Blood (BF), Periodontal (PCF) and calibration curve)

The periopaper® with the interleukins was extracted (by methodology stated above for the animal model) and the collected fluid displayed in the ELISA kits as shown in table 39 to 42 respectively for IL6 and IL-1β.

Table 39 and 40 show, an ELISA plate and the respective wells for IL6 and table 41 and 42 for IL-1β. The result in each well represents an optical density found for one sample.

Table 40A - Plaque 1: ELISA IL6 Reading Peri-implant/Periodontal/Blood and Calibration Curve Samples Express in Optical Densities (OD)

3,877	1,523	0,275	0,103	0,206	0,227	0,411	0,160	0,122	0,087	0,098
3,144	0,634	0,477	0,106	0,274	0,099	0,140	0,135	0,110	0,189	0,177
3,392	0,858	0,228	0,078	0,150	0,101	0,337	0,100	0,103	0,118	0,132
2,202	0,783	0,210	0,074	0,187	0,138	0,083	0,189	0,208	0,206	0,234
1,200	0,785	0,207	0,054	0,172	0,231	0,118	0,138	0,117	0,098	0,163
1,414	0,422	0,197	-	0,189	0,323	0,166	0,087	0,121	0,164	0,133
0,044	0,506	0,212	-	0,180	0,611	0,350	0,358	0,120	0,108	0,133
0,700	0,646	0,129	0,182	0,205	0,111	0,230	0,137	0,170	0,127	0,116

Table 40B - Plaque 2: ELISA IL6 Reading Peri-implant/Periodontal/Blood and Calibration Curve Samples Express in Optical Densities (OD)

0,092	0,091	0,111	0,072	0,063	0,106	0,061	0,102	0,056	0,069	0,080
0,215	0,094	0,234	0,114	0,150	0,163	0,113	0,086	0,068	0,063	0,083
0,079	0,640	0,125	0,095	0,056	0,075	0,250	0,091	0,083	0,078	X
0,114	0,166	0,177	0,224	0,132	0,104	0,086	0,084	0,069	0,092	X
0,048	0,106	0,081	0,066	0,145	0,099	0,082	0,080	0,071	0,059	X
0,251	0,183	0,192	0,121	0,083	0,072	0,081	0,076	0,071	0,080	X
0,120	0,124	0,102	0,069	0,101	0,074	0,091	0,082	0,080	0,082	X
0,072	0,173	0,127	0,143	0,106	0,098	0,080	0,084	0,063	0,061	

Table 41 - Plaque 1: ELISA IL-1β Reading Peri-implant/Periodontal/Blood and Calibration Curve Samples expressed in Optical Densities (O.D)

3,799	1,05	0,501	0,271	0,407	2,832	0,823	0,636	0,710	0,439	3,709
3,453	1,966	0,485	0,108	0,253	0,144	0,118	0,339	0,127	0,317	0,204
3,492	0,898	0,423	0,175	0,288	0,265	3,632	1,294	0,147	2,674	0,269
3,823	0,600	0,221	0,167	0,172	0,303	0,137	0,121	0,477	0,425	0,343
2,438	0,856	0,208	0,111	0,157	2,281	1,012	0,232	2,975	3,593	0,314
2,819	0,574	0,360	0,071	0,202	0,190	0,200	0,156	0,111	0,155	0,142
2,423	0,400	0,204	0,094	0,254	1,384	3,132	2,432	0,282	0,656	1,581
1,567	0,420	0,175	0,191	0,159	0,109	0,264	0,124	0,121	0,172	0,164

Table 42 - Plaque 2: ELISA IL-1β Reading Peri-implant/Periodontal/Blood and Calibration Curve Samples expressed in Optical Densities (O.D)

0,362	1,522	1,267	0,261	0,747	0,176	O,234	0,113	0,241	0,153	0,631
0,128	0,088	0,255	0,162	0,541	0,227	0,186	0,139	0,620	0,137	0,227
0,394	0,681	1,363	0,711	0,380	0,324	0,234	0,109	0,606	1,309	0,259
0,158	0,203	0,200	0,164	0,113	0,197	0,110	0,810	0,269	0,860	X
0,299	2,611	0,396	0,939	0,632	0,160	0,124	0,129	0,276	0,217	X
0,213	0,277	0,186	0,237	0,099	0,269	0,095	0,102	0,161	0,391	X
0,742	3,343	1,057	1,168	0,735	0,419	0,102	0,105	0,681	0,996	X
0,155	0,316	0,165	0,365	0,115	0,112	0,221	0,377	0,165	0,234	X

Results for Calibration Curve Formation

To transfer the results in optical densities to concentration levels an equation that best fits the curve must be determined. To do this. a calibration curve was plotted based on the results obtained.

Calibration Curve for IL6

With the concentrations determined by the Elisa kit Supplier the aliquots that had previous been prepared were read.

For each calibration point several duplicates were read in order to achieve the best curve possible. As shown in table 43, for some points, the calibration point was read five times.

One calibration curve was created for each interleukin (1β and 6). Table 43 represents the concentration levels predetermined by the supplier, where R1 to R7 referes to the optical densities found for each concentration sample. 1 to 7 represent the amount of times that a sample was read. Each concentration point was averaged, and the mean was plotted to the final calibration curve.

On the calibration curve, we could see that each point had a mean and a standard deviation and once the curve is set then the optical densities samples could be plotted to the curve, and a concentration value found.

On the Excel (2008) sheet the best equation is determined, and all the values are plotted onto a graphic.

Table 43 - Results for IL6 calibration curve readings at different time frames. Mean average and standard deviation

	IL6 Standards									
[]	R1	R2	R3	R4	R5	R6	R7	Mean	Mean 0	Deviation
1000	3,877							3,877	3,808	-
300	3,144				3,392			3,392	3,323	-
150	2,202	1,2			1,414			1,307	1,238	0,151
75	0,044	0,7	1,523	0,634	0,858	0,783		0,744	0,675	0,098
37,5	0,785	0,422			0,506			0,464	0,395	0,059
18,75	0,646	0,275			0,477	0,228		0,327	0,258	0,132
9,375		0,21		0,207	0,197			0,205	0,136	0,007
4,69	0,212	0,129			0,103			0,116	0,047	0,018
0	0,106	0,078			0,074		0,054	0,069	0,000	0,013

[] Predetermined IL6 Concentration from ELISA kit

R (1-7)– Different time frames from which data for the calibration curve was collected

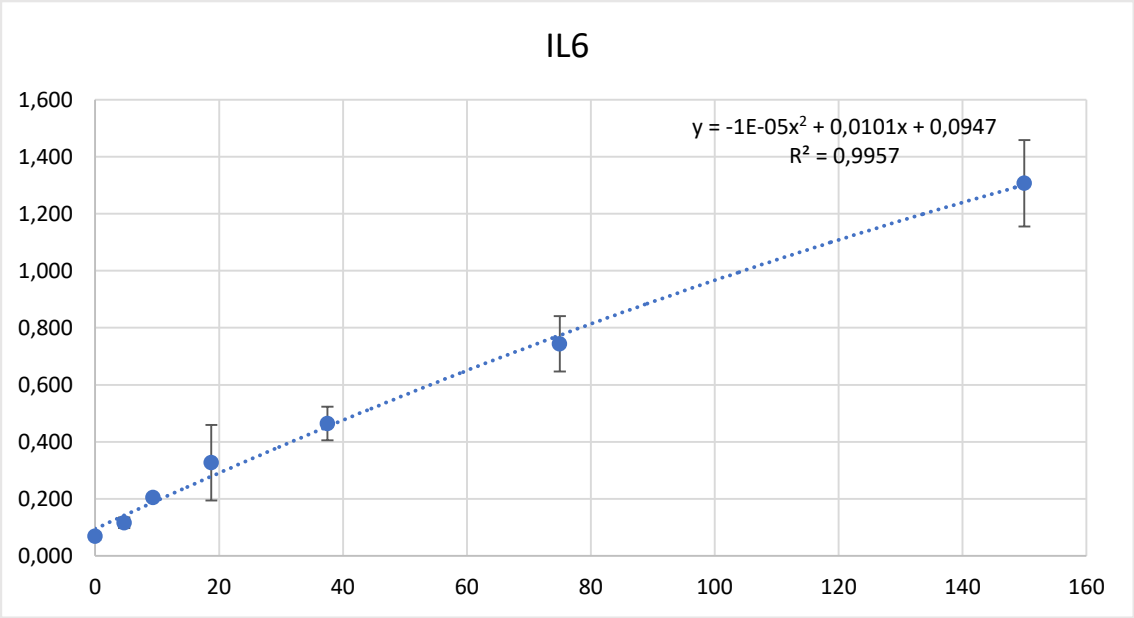


FIGURE 118 - Calibration Curve results for IL6. Each point is the result of several readings at different time frames (mean and standard deviation). The outcome results in a polynomial equation that allows reading from Optical Densities (OD) to Concentration Levels [ ]

Calibration Curve for IL-1β

The same methodology was applied to IL-1β where the concentration levels were plotted**.** The results are displayed in table 44 and again, the duplicates were averaged to achieve a final value for plotting on a graphic.

Fig. 119 shows the calibration curve obtained for IL-1β and again it is not a linear proportional curve, so a polynomial equation was generated for reading concentrations.

Table 44 - Results for IL-1β calibration curve readings at different time frames. Mean average and standard deviation

Standards IL-1β										
[]	R1	R2	R3	R4	R5	R6	R7	Mean	0 Mean	Deviation
500							3.799	3.799	-	-
250,00	3,453				3,492		3,823	3,658	3,575	0,234052345
125,00	2,438				2,819		2,423	2,621	2,539	0,280014285
62,50	1,567	1,05			1,966			1,508	1,426	0,647709812
31,20	0,898	0,6			0,856			0,728	0,646	0,181019336
15,60	0,574	0,4		0,42	0,501	0,485		0,452	0,369	0,049047596
7,80	0,423	0,221	0,208		0,36			0,263	0,181	0,084255564
3,90	0,204	0,175			0,271			0,223	0,141	0,067882251
1,95		0,108		0,175	0,167			0,150	0,068	0,036592349
0,00	0,111	0,071			0,094			0,083	0,000	0,016263456

[] Predetermined IL6 Concentration from ELISA kit

R (1-7)– Different time frames from which data for the calibration curve was collected

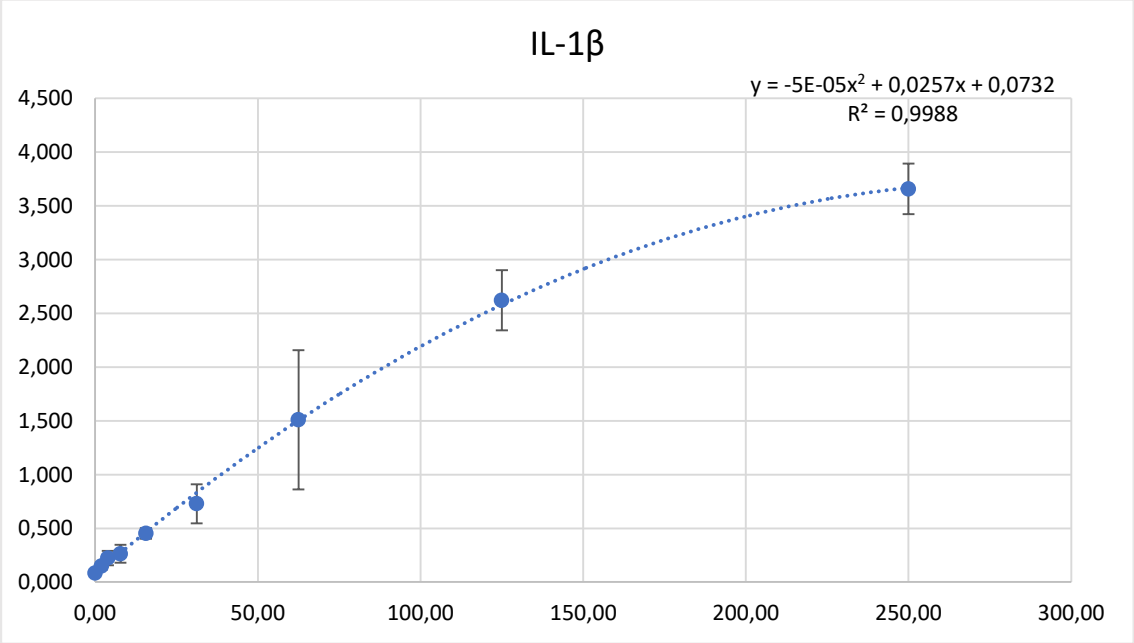


FIGURE 119 - Calibration Curve results for IL-1β. Each point is the result of several readings at different time frames (mean and standard deviation). The outcome results in a polynomial equation that allows reading from Optical Densities (OD) to Concentration Levels []

Elisa Plate Reading in Concentration (pg/ml)

After the calibration curve was set, the optical densities were plotted from each sample reading both for IL-1β and IL6. All samples were inserted in the polynomial equation for each interleukin and a table was obtained for final concentration values.

For IL6 according to the calibration curve the following equation was obtained:

$$y = -1E\text{-}05x2 + 0{,}0101x + 0{,}0947$$

$$R^2 = 0{,}99569$$

From this, all samples obtained a concentration value.

The IL6 values are displayed on table 45 in pg/ml, divided by time frames (T) and by material (Z, A, T).

Table 45 - IL6 General obtained Optical Densities (OD) and the corresponding concentrations values in pg/ml at T0 baseline and T2 (8weeks) in each material (Z, A, T). Polynomial result is also displayed

		IL6		IL6		IL6 (T0)	IL6 (T2)
		Optical Densities (OD)		pg/ml			
Material	Patient nº	T0	T2	T0	T2	Polinomial	Polinomial
A	1	0,182	0,206	9	11	-0,000134026	-0,000383224
A	2	0,274	0,15	18	5	-0,000620486	-9,50892E-05
A	3	0,187	0,172	9	8	-0,000176454	-6,41546E-05
A	8	0,323	0,611	23	54	-1,82459E-05	-0,000797764
T	5	0,205	0,227	11	13	-0,000370544	-3,89377E-05
Z	4	0,189	0,18	9	9	-0,000194823	-0,000118454
Z	13	0,23	0,16	14	7	-6,26164E-05	-6,67779E-06
Z	16	0,189	0,138	9	4	-0,000194823	-3,32869E-05
A	9	0,111	0,411	2	32	-9,6393E-07	-9,39652E-06
Z	21	0,11	0,103	2	1	-7,1801E-05	-5,75905E-07
A	11	0,083	0,118	-1	2	-3,79211E-06	-1,28228E-05
A	12	0,166	0,35	7	26	-3,18203E-05	-5,65791E-05
T	6	0,099	0,101	0	1	-3,66067E-07	-1,61386E-06
Z	24	0,121	0,12	3	3	-2,0859E-05	-1,79833E-05
T	7	0,138	0,231	4	14	-3,32869E-05	-7,09166E-05
T	10	0,14	0,337	5	25	-4,16087E-05	-2,57032E-05
Z	25	0,17	0,087	8	-1	-5,25774E-05	0,000523959
Z	29	0,127	0,098	3	0	-0,000985721	-0,000169961

A	27	0,206	0,098	11	0	-0,000383224	-0,000169961
Z	33	0,116	0,092	2	0	-8,44992E-06	-4,04717E-07
T	14	0,135	0,1	4	1	-2,22884E-05	-8,91853E-07
Z	34	0,215	0,079	12	-2	-0,000506339	0,000426199
Z	38	0,094	0,64	0	57	0,0007	-1,26542E-05
T	17	0,087	0,358	-1	27	0,000523959	-0,000175772
T	20	0,137	0,122	4	3	-2,94229E-05	-2,39316E-05
A	30	0,177	0,132	8	4	-9,65934E-05	-1,30705E-05
Z	40	0,183	0,124	9	3	-0,000142112	-3,06675E-05
Z	41	0,173	0,111	8	2	-7,02428E-05	-9,6393E-07
T	23	0,208	0,117	11	2	-0,000409185	-1,05379E-05
T	26	0,189	0,118	9	2	-0,000194823	-1,28228E-05
A	45	0,127	0,072	3	-2	-0,000985721	-1,12517E-05
Z	42	0,234	0,125	14	3	-9,70392E-05	-3,43309E-05
A	36	0,251	0,12	16	3	-0,000279689	-1,79833E-05
Z	43	0,177	0,081	8	-1	-9,65934E-05	-6,74506E-06
Z	46	0,114	0,095	2	0	-4,86475E-06	-0,0003
A	39	0,166	0,106	7	1	-3,18203E-05	-3,31764E-06
Z	54	0,106	0,106	1	1	-3,31764E-06	-3,31764E-06
T	28	0,164	0,108	7	1	-2,26404E-05	-6,12775E-06
T	31	0,234	0,163	14	7	-9,70392E-05	-1,83501E-05
T	32	0,133	0,133	4	4	-1,59453E-05	-1,59453E-05
T	35	0,114	0,058	2	-4	-4,86475E-06	-1,08068E-05
A	44	0,192	0,102	10	1	-0,000223875	-0,000134041
T	37	0,072	0,091	-2	0	-1,12517E-05	0,000568039
A	47	0,224	0,066	13	-3	-1,70919E-05	-2,8205E-05
A	50	0,143	0,063	5	-3	-5,55752E-05	0,000192279
Z	55	0,163	0,075	7	-2	-1,83501E-05	-5,41032E-06
A	52	0,132	0,145	4	5	-1,30705E-05	-6,58756E-05
Z	59	0,098	0,061	0	-3	-0,000169961	-3,92396E-06
T	49	0,121	0,069	3	-3	-2,0859E-05	-1,88499E-05
T	51	0,15	0,056	5	-4	-9,50892E-05	-1,63667E-05
A	57	0,104	0,099	1	0	-1,29336E-06	-3,66067E-07
A	58	0,072	0,074	-2	-2	-1,12517E-05	-7,16225E-06
T	53	0,083	0,101	-1	1	-3,79211E-06	-1,61386E-06
A	60	0,113	0,215	2	12	-3,36755E-06	-0,000506339

The same rationale was used for the control groups BF and PCF levels.

The Elisa plates with the respective well order are displayed in table 46 and 47.

Table 46 - Plaque 1: ELISA IL6 readings of Peri-implant/Periodontal/Blood and Calibration Curve Samples expressed in concentration levels (pg/ml)

n/d	n/d	n/d	n/d	11	13	32	7	3	-1	0
n/d	n/d	n/d	n/d	18	0	5	4	2	9	8
n/d	n/d	n/d	n/d	5	1	25	1	1	2	4
n/d	n/d	n/d	n/d	9	4	-1	9	11	11	14
n/d	n/d	n/d	n/d	8	14	2	4	2	0	7
n/d	n/d	n/d	n/d	9	23	7	-1	3	7	4
n/d	n/d	n/d	n/d	9	54	26	27	3	1	4
n/d	n/d	n/d	9	11	2	14	4	8	3	2

Table 47 - Plaque 2: ELISA IL6 readings of Peri-implant/Periodontal/Blood and Calibration Curve Samples expressed in concentration levels (pg/ml)

0	0	2	-2	-3	1	-3	1	-4	-3	-1
12	0	14	2	5	7	2	-1	-3	-3	-1
-2	57	3	0	-4	-2	12	0	-1	-2	X
2	7	8	13	4	1	-1	-1	-3	0	X
-4	1	-1	-3	5	0	-1	-1	-2	-4	X
16	9	10	3	-1	-2	-1	-2	-2	-1	X
3	3	1	-3	1	-2	0	-1	-1	-1	X
-2	8	3	5	1	0	-1	-1	-3	-3	X

For IL-1β according to the calibration curve the following equation was obtained:

$$y = -5E\text{-}05x^2 + 0{,}0257x + 0{,}0732$$

$$R^2 = 0{,}99876$$

In table 48 the final concentrations of all the samples are displayed by time frame and material.

Table 48 - IL-1β General obtained Optical Densities (OD) and the corresponding concentrations values in pg/ml at T0 baseline and T2 (8weeks) in each material (Z, A, T). Polynomial result is also displayed

		IL-1β Optical Densities (OD)		IL-1β Pg/ml		IL-1β T0	IL-1β T2
Material	Patient nº	T0	T2	T0	T2	Polynomial	Polynomial
A	1	0,191	0,407	5	13	-0,000233884	-0,000215859
A	2	0,253	0,288	7	8	-2,01587E-05	-0,000580806
A	3	0,172	0,157	4	3	-8,9681E-05	-1,51474E-05
A	8	0,19	1,384	5	57	-0,000224907	-0,000142722
T	5	0,159	2,832	3	153	-2,30811E-05	-2,13878E-05
Z	4	0,202	0,254	5	7	-0,000342807	-0,000164207
Z	13	0,264	0,636	8	23	-0,000267883	-0,000171094
Z	16	0,121	0,232	2	6	-2,05689E-05	-0,000734679
A	9	0,109	0,823	1	31	-3,71911E-05	-5,8432E-05
Z	21	0,127	0,147	2	3	-4,10417E-05	-0,000148992
A	11	0,137	1,012	2	40	-8,73807E-05	-0,000540224
A	12	0,2	3,132	5	187	-0,000353634	-0,000907416
T	6	0,144	0,265	3	8	-0,000128905	-0,000279115
Z	24	0,111	0,282	1	8	-4,43986E-05	-0,000494092
T	7	0,303	2,281	9	109	-0,000822338	-0,000931191
T	10	0,118	3,632	2	-	-1,23942E-05	-3,5588
Z	25	0,121	0,439	2	15	-2,05689E-05	-0,000792771
Z	29	0,172	3,709	4	-	-8,9681E-05	-3,6358
A	27	0,425	3,593	14	-	-0,000519801	-3,5198
Z	33	0,164	0,362	4	11	-4,56132E-05	-2,20939E-05
T	14	0,339	1,294	11	53	-1,32135E-05	-1,67979E-05
Z	34	0,128	0,394	2	13	-4,49884E-05	-3,9925E-05
Z	38	0,088	0,681	1	25	-6,20805E-06	-0,000268808
T	17	0,156	2,432	3	120	-1,14118E-05	-3,13066E-05
T	20	0,124	0,71	2	26	-3,01181E-05	-0,000386066
A	30	0,204	0,269	5	8	-0,000364616	-0,000325613
Z	40	0,277	3,343	8	231	-0,00042615	-0,000233358
Z	41	0,316	1,267	10	52	-7,06163E-06	-1,35287E-05
T	23	0,477	2,975	16	167	-1,22762E-05	-0,000132689
T	26	0,317	2,674	10	138	-7,27904E-06	-0,00047596
A	45	0,165	0,261	4	7	-5,05821E-05	-0,00023513

Z	42	0,255	1,363	7	56	-0,000173867	-9,61975E-05
A	36	0,213	0,742	5	27	-0,000470384	-6,08853E-06
Z	43	0,2	0,396	5	13	-0,000321616	-5,61565E-05
Z	46	0,162	0,711	3	26	-3,61379E-05	-0,000415484
A	39	0,203	2,611	5	133	-0,000353634	-7,04571E-05
Z	54	0,115	0,176	2	4	-5,59392E-06	-0,000115415
T	28	0,155	0,656	3	24	-7,83034E-06	-0,000210923
T	31	0,343	0,314	11	10	-1,4548E-05	-6,63947E-06
T	32	0,142	1,581	3	68	-0,000116277	-5,14336E-06
T	35	0,158	0,299	3	9	-1,90372E-05	-0,000754473
A	44	0,186	1,057	4	42	-0,000190541	-0,000762758
T	37	0,155	1,524	3	65	-7,83034E-06	-0,000778085
A	47	0,164	0,939	4	36	-4,56132E-05	-0,000277908
A	50	0,365	0,747	12	28	-2,34794E-05	-7,60659E-06
Z	55	0,227	0,324	6	10	-0,000659732	-8,92271E-06
A	52	0,113	0,632	2	23	-1,82401E-06	-0,00016378
Z	59	0,112	0,234	2	6	-0,000107005	-0,000765737
T	49	0,237	1,168	6	47	-2,40134E-05	-5,65094E-06
T	51	0,541	0,38	19	12	-4,80323E-05	-3,12687E-05
A	57	0,197	0,16	5	3	-0,000290984	-2,72792E-05
A	58	0,269	0,419	8	14	-0,000325613	-0,00041261
T	53	0,099	0,735	1	27	-1,02739E-05	-4,27451E-06
A	60	0,186	0,234	4	6	-0,000190541	-0,000765737

The same rationale for the control group was used for the IL-1β. Both the BF and the PCF were read and table 49 and 50 show the final concentration results.

Table 49 - Plaque 1: ELISA IL-1β Reading Peri-implant/Periodontal/Blood and Calibration Curve Samples expressed in concentration (pg/ml)

n/d	n/d	n/d	n/d	13	153	31	23	26	15	-
n/d	n/d	n/d	n/d	7	3	2	11	2	10	5
n/d	n/d	n/d	n/d	8	8	-	53	3	138	8
n/d	n/d	n/d	n/d	4	9	2	2	16	14	11
n/d	n/d	n/d	n/d	3	109	40	6	167	-	10
n/d	n/d	n/d	n/d	5	5	5	3	1	3	3
n/d	n/d	n/d	n/d	7	57	187	120	8	24	68
n/d	n/d	n/d	5	3	1	8	2	2	4	4

Table 50 - Plaque 2: ELISA IL-1β Reading Peri-implant/Periodontal/Blood and Calibration Curve Samples expressed in concentration (pg/ml)

11	65	52	7	28	4	6	2	7	3	23
2	1	7	3	19	6	4	3	22	2	6
13	25	56	26	12	10	6	1	22	54	7
3	5	5	4	2	5	1	30	8	33	X
9	133	13	36	23	3	2	2	8	6	X
5	8	4	6	1	8	1	1	3	13	X
27	231	42	47	27	14	1	1	25	39	X
3	10	4	12	2	2	6	12	4	6	X

The overall available samples for concentration level readings are shown in fig. 101. Every sample studied at T0 was able to be studied at T2. With the exception of 2 samples of IL-1β at T2 they exceed the concentration capacity of the Elisa test to determine a value and were thus discarded.

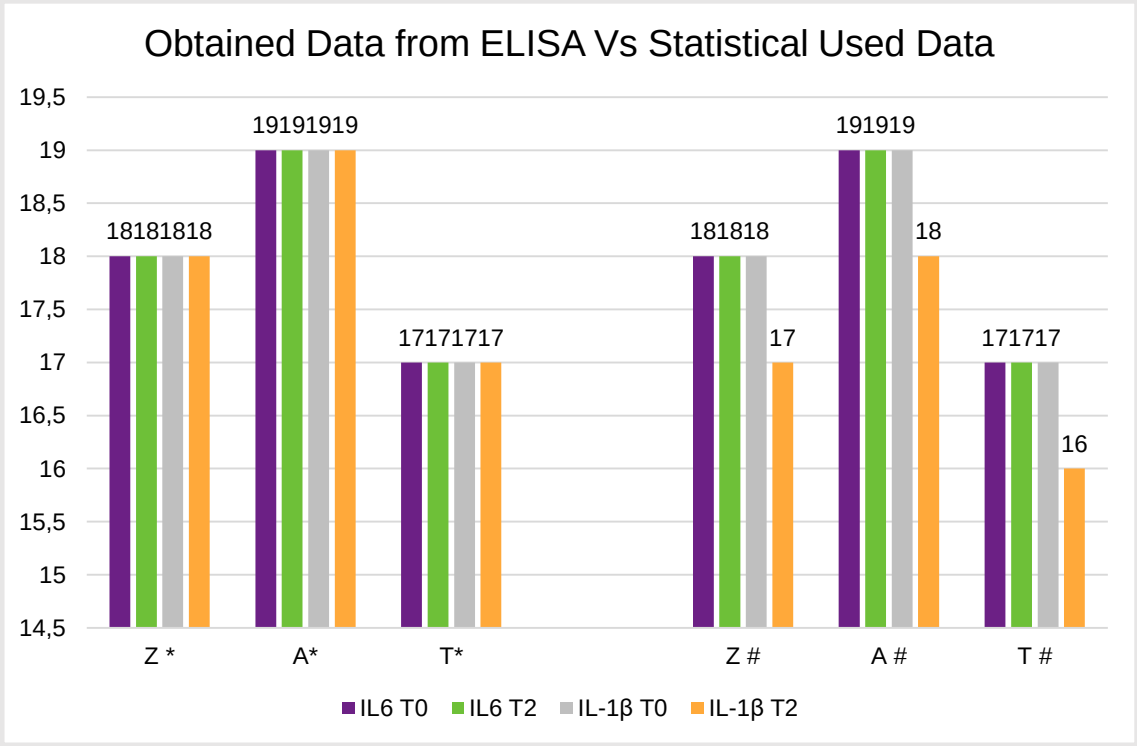


FIGURE 120 - N samples for different time frames of the laboratory procedures and statistical analysis *Data used for IL-1β and IL6 Elisa Readings # data available for statistical comparisons. The results are different due to IL-1β at T2 samples exceeding the permitted Elisa Interleukin concentration and thus not yielding an accurate result.

On the statistical level, to relate quantitative variables (such as IL6, IL-1β and total inflammation) to qualitative variables (such as the material or moment, T0 and T2), the process was as follows:

When there were two cases (moments) in the qualitative variable, the parametric test T was used, or if the quantitative variable had a normal distribution and the samples in each of the two groups were large (more than 30). If these assumptions do not exist, then the non-parametric alternative Mann-Whitney test was used.

When there were 3 or more cases (material) in the qualitative variable, the parametric ANOVA test was used, or if the quantitative variable had a normal distribution and the samples in each of the groups were large (more than 30) and there was homogeneity of the variances. If these assumptions did not exist, then the nonparametric alternative Kruskall-Wallis test was used.

In this study for all variables the significance level of 5% ($p \leq 0,05$) was considered adequate. When the *p*-value of the test in question was less than

0,05, the null hypothesis of said test was rejected.

Overall Interleukin variation between T0 and T2 (independent of the healing abutment material)

Table 51 - Overall IL6 and IL-1β concentrations at T0 and T2

T	IL6 pg/ml	IL-1β pg/ml	IL6+ IL-1β pg/ml
T0	6,20±5,43	5,24±3,91	11,44±7,62
T2	6,01±12,58	55,41±49,85	47,27±53,22

For the overall difference between implants placed at T0 and implants analyzed at T2 independently of the material, 54 implants were analyzed for IL6 and 51 for IL-1β and in total (IL-1β and IL6). The overall IL6 and IL-1β concentrations at T0 and T2 are displayed in table 51

Table 52 - Mean and standard deviation (SD) of Concentrations in pg/ml of Total interleukins from T0 to T2 (difference) independent of material

T	IL6 pg/ml	IL-1β pg/ml	IL6+IL-1β pg/ml
T0-T3 (Diff)	-0,18±12,98	36,23±48,86	35,92±51,89

The mean average of the difference found for IL6 was -0,18±12,98 pg/ml, for IL-1β 36,23 ± 48,86 pg/ml and for the total 35,92 ± 51,89 pg/ml as shown in table 52.

The sample was large enough for a T-test for significance ($p \leq 0,05$) to analyze the differences between variables. It was verified that these differences were significantly dissimilar to 0, that IL-1β and in total, differed significantly between T0 and T2, independently of the material.

Only in IL6 the difference between T0 and T2 was not significantly dissimilar to 0, that is, between T0 and T2, there were no significant differences in IL6.

For IL-1β and total interleukin concentration (IL-1β+IL6), the difference was significantly dissimilar to 0 (*p*-value = 0,000). As a result of this positive difference, IL-1β at T2 - IL-1β at T0> 0, so IL-1β was significantly higher at T2. The same reasoning was used for total interleukin (IL-1β + IL6) as shown in table 53.

Table 53 - Hypothesis and statistical conclusions of the Overall Difference of IL6, IL-1β and Total IL, between T0 and T2

Interleukin	Test	Null Hypothesis	*P*-Value
IL6	T-test	Retain	0,917
IL-1β	T-test	Reject	0,00
IL6 + IL-1β	T-test	Reject	0,00

At T0 (Baseline) Implant Placement (IL6, IL-1β and Total IL-1β+IL6)

One of the main pillars of this investigation was to observe the behavior of the inflammation pattern at different time frames.

At T0 concentration values of IL6, IL-1β and in total (IL-1β+IL6) were obtained from each material, cad-cad zirconia, cad-cam titanium and cad-cam acrylic.

For the 17 titanium (T) abutments measured, the mean average of IL6 (pg/ml) found was 4,65 pg/ml with a SD of 4,57 pg/ml. For IL-1β (pg/ml) the mean concentration found was 6,35 pg/ml with a SD deviation of 5,37 pg/ml and for the total (IL6+IL-1β) the mean average was 11 pg/ml with a SD deviation of 8,59 pg/ml.

For the 19 Acrylic (A) abutments measured, the mean average for IL6 concentrations was 7,63 pg/ml with a SD deviation of 6,58 pg/ml. For IL-1β the mean was 5,31 pg/ml with a SD deviation of 3,16 while for the total (IL-1β+IL6) the mean average was 12,95 with a SD deviation of 7,78 pg/ml.

For the 18 Zirconia (Z) abutments measured the mean average of IL6

concentrations was 6,12 pg/ml with a SD deviation of 4,64 pg/ml. For IL-1β the mean was 4,11 pg/ml with SD deviation of 2,7 pg/ml while for the total (IL-1β +IL6) the mean average was 10,28 pg/ml with a SD deviation of 6,6 pg/ml, as shown in table 54 and in fig. 102.

Table 54 - Mean and standard deviation of Concentration in pg/ml of Interleukins at T0 Baseline

M	IL6	IL-1β	IL6+IL-1β
Z	6,17 ± 4,64	4,11 ± 2,7	10,28 ± 6,6
A	7,63 ± 6,58	5,31 ± 3,16	12,95 ±7,78
T	4,65 ± 4,57	6,35 ± 5,37	11 ± 8,59

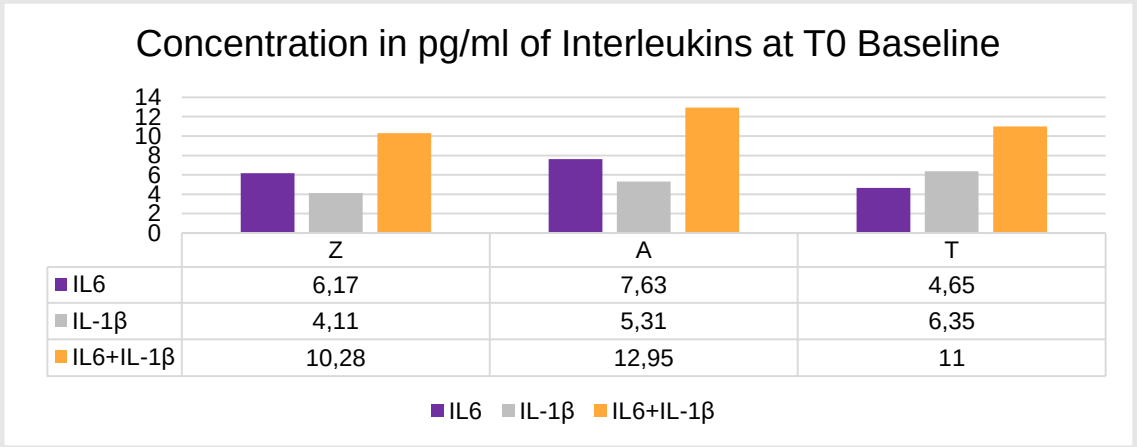


FIGURE 121 - Concentration in pg/ml of Interleukins at T0 Baseline. Zirconia, Acrylic and Titanium Healing abutment (Z, A, T) IL6, IL-1β and total interleukin displayed.

The sample size was different in each IL so different statistical tools were tailored to fit this, as shown in table 55.

Table 55 - Hypothesis and statistical conclusions of IL6, IL-1β and IL6+IL-1β and the relationship with different biomaterials (Zirconia, Acrylic and Titanium) at T0

Interleukin	Biomaterial	Test	Null Hypothesis	P-Value
IL-1β	A	kruskall-Wallis	retain	0,337
	Z			
	T			
IL6	A	Anova	retain	0,262
	Z			
	T			
IL-1β +IL6	A	Anova	retain	0,553
	Z			
	T			

The statistical analysis was completed and for IL6 and in total (IL-1β+IL6), the variables satisfied the ANOVA assumptions. In these cases, *p*-values were greater than 0,05 $p \leq 0,05$ (p=0,262 for IL6 and p=0,553 for IL6+IL-1β, respectively). Thus, at T0, IL6 and in total (IL6+ IL-1β) did not differ significantly with the material (Z, T or A).

IL-1β did not satisfy the ANOVA assumptions, so the non-parametric Kruskall-Wallis test was used, which led to a *p*-value of 0,337> 0,05 (below). In addition, at T0, the IL-1β did not differ significantly with the material (Z, T or A).

We can see graphically in the boxplots (fig. 103-105) the variations by quarter found in the different IL and in relation to the different material.

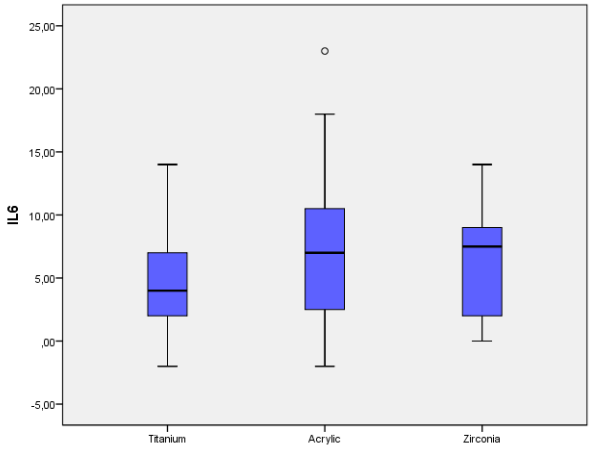


FIGURE 122 - Boxplot of IL6 results for T, A and Z at T0.

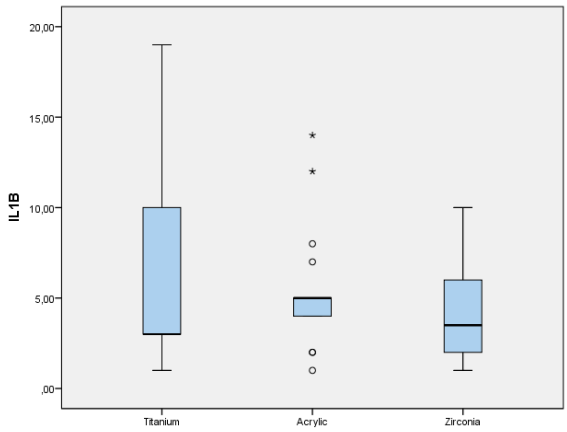


FIGURE 123 - Boxplot of IL-1β results for T, A and Z at T0.

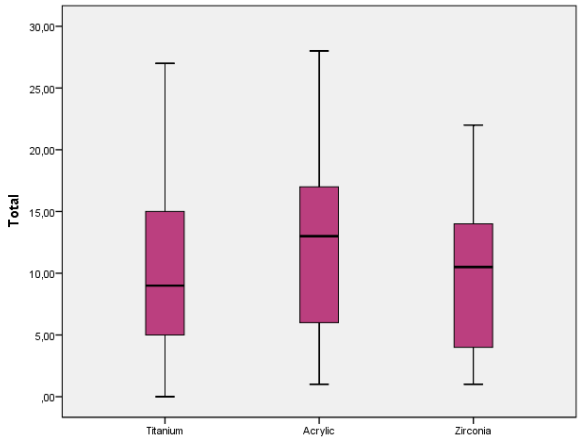


FIGURE 124 - Boxplot of IL-1β + IL6 results for T, A and Z at T0.

At T2 (two months after T0) Osseointegration (IL6, IL-1β and Total IL-1β +IL6)

T0 baseline was followed by a period of 8 weeks after which the the inflammation pattern of each healing abutment was analyzed. The mean average of IL6 found on Zirconia (Z) was 4,76 ± 13,83 pg/ml for Acrylic (A) 8,56 ± 14,82 pg/ml and 4,06 ± 7,99 pg/ml for Titanium (T).

For IL-1β the mean values for Zirconia were 29,94 ± 54,07 pg/ml for Acrylic 31,44 ± 33,40 pg/ml and 64,75 ± 55,24 pg/ml for Titanium.

For the Total a mean average of 34,70 ± 55,99 pg/ml for Zirconia 40 ± 39,66 pg/ml for Acrylic and 68,81 ± 59,81 pg/ml for Titanium, as shown in table 56 and fig. 106.

Table 56 - Mean and standard deviation of Concentration in pg/ml of Interleukins at T2 (eight weeks)

Material	IL6	IL-1β	IL6+IL-1β
Z	4,76 ± 13,83	29,94 ± 54,07	34,70 ± 55,99
A	8,56 ± 14,82	31,44 ± 33,40	40 ± 39,66
T	4,06 ± 7,99	64,75 ± 55,24	68,81 ± 59,81

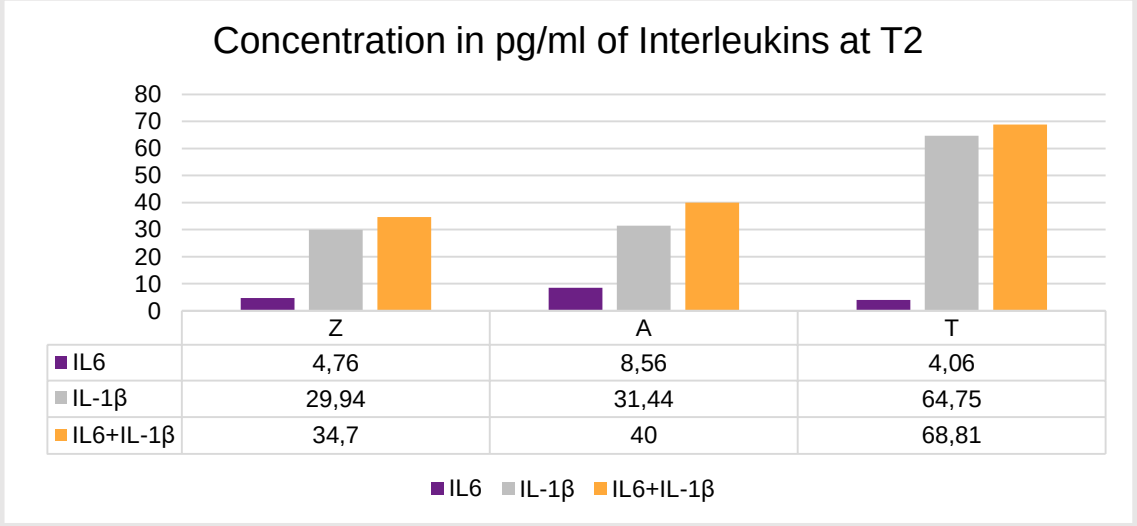


FIGURE 125 - Concentration in pg/ml of Interleukins at T2 (8 weeks). Zirconia, Acrylic and Titanium Healing abutment (Z, A, T) IL6, IL-1β and total interleukin display.

The sample size was different in each IL so different tools were designed, as shown in table 57.

Table 57 - Hypothesis and statistical conclusions of IL6, IL-1β and IL6+IL-1β and the relationship to different biomaterials (Zirconia, Acrylic and Titanium at T2

Interleukin	Biomaterial	Test	Null Hypothesis	*P*-Value
IL-1β *	A -T	Kruskall-Wallis	retain	0,191
	Z-A		retain	1,000
	T-Z		Reject	0,023
IL6	A	Kruskall-Wallis	retain	0,561
	Z			
	T			
IL-1β +IL6	A	Kruskall-Wallis	retain	0,069
	Z			
	T			

*a pair to pair comparison was done to see which sets of material was statistical different

A-T – Acrylic-Titanium

Z-A – Zirconia-Acrylic

T-Z – Titanium-Zirconia

As a result, none of the three variables satisfied the ANOVA assumptions, so the Kruskall-wallis test was used.

Only IL-1β differed at T2, significantly, with the material (*p*-value = 0,025). Analyzing the pairwise comparisons at T2, IL-1β differed significantly between titanium and zirconium, with IL-1β being, on average, significantly higher in the titanium (*p*-value = 0,023). There was no significant difference in the other 2 pairs (zirconia-acrylic and titanium acrylic).

We can see the variations by quarter found in the different IL and the relation to the different materials graphically in the boxplots (fig. 107-109).

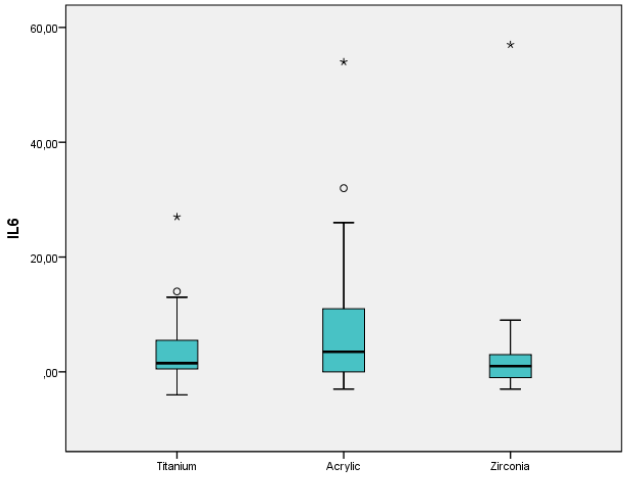


FIGURE 126 - Boxplot of IL6 results for T, A and Z at T2

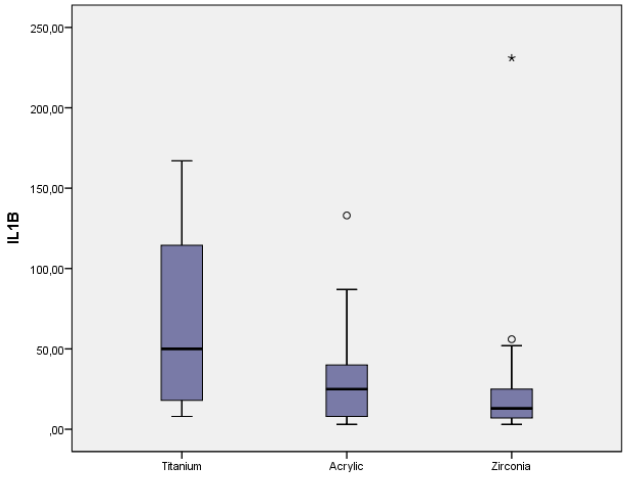


FIGURE 127 - Boxplot of IL-1β results for T, A and Z at T2

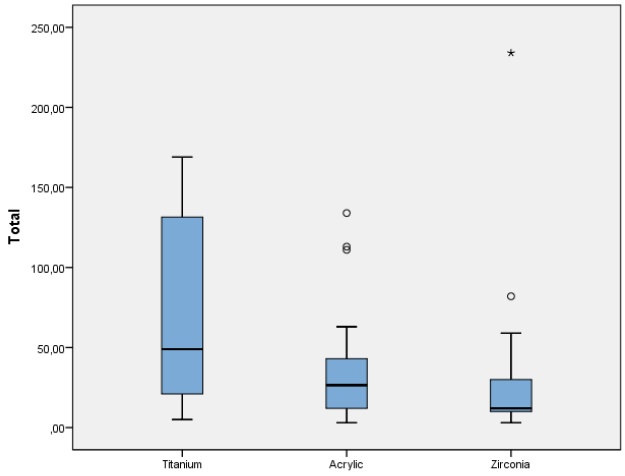


FIGURE 128 - Boxplot of IL6+IL-1β (Total) results for T, A and Z at T2

Interleukin Concentration in each material and their variation from T0 to T2

As in the previous calculation for the overall result, the same assumptions were used for each individual material for calculate IL concentrations differences between T0 and T2.

The mean average and SD for each material is shown in table 58, as displayed graphically in fig. 110.

Table 58 - Mean and Standard Deviation of Concentration in pg/ml of Interleukins at T0 Baseline and at T2 in each material (Z, A, T)

Material	IL6 T0	IL6 T2	IL-1β T0	IL-1β T2	IL6+ IL-1β T0	IL6+ IL-1β T2
Z	6,12	4,76	4,11	29,94	10,28	34,70
	± 4,64	± 13,83	± 2,7	± 54,07	± 6,6	± 55,99
A	7,63	8,56	5,31	31,44	12,95	40
	± 6,58	± 14,82	± 3,16	± 33,40	±7,78	± 39,66
T	4,65	4,06	6,35	64,75	11	68,81
	± 4,57	± 7,99	± 5,37	± 55,24	± 8,59	± 59,81

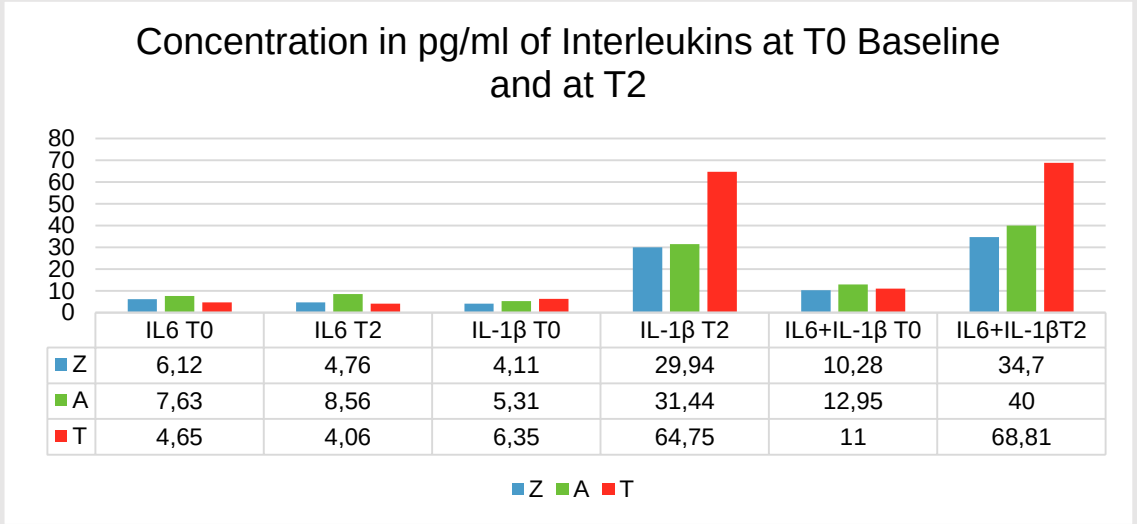


FIGURE 129 - Concentration in pg/ml of Interleukins at T0 (Baseline) and at T2 (8 weeks). Zirconia, Acrylic and Titanium Healing abutment (Z, A, T) IL6, IL-1β and total interleukin display

Interleukin Analysis by material

There were only a few elements in each group and therefore the T-test could only be used if the distribution was normal. Alternatively, to check whether the

difference was significantly different, a non-parametric test was used which, in this case, was the signals test.

Titanium and Interleukin Variation

The titanium healing abutment was analyzed, and the different IL concentrations were observed to see if there was a pattern from T0 to T2.

The mean average and SD found are shown in table 59 and fig. 101.

There was not much variation in IL6 from T0 to T2 indeed the mean average value was lower at T2 than at T1, but IL-1β experienced a significant increase in concentration from T0 to T2.

There was an overall increase in IL6+IL-1β concentration primarily associated with the IL-1β increase.

Table 59 - Titanium Healing Abutment (T) Mean Average and Standard Deviation of IL-1β, IL6 and total Interleukin over different time frames (T0 to T2)

	IL6	IL-1β	IL6+ IL-1β
T0	4,65±4,57	6,35±5,37	11±8,59
T2	4,06±7,99	64,75±55,24	68,81±59,81

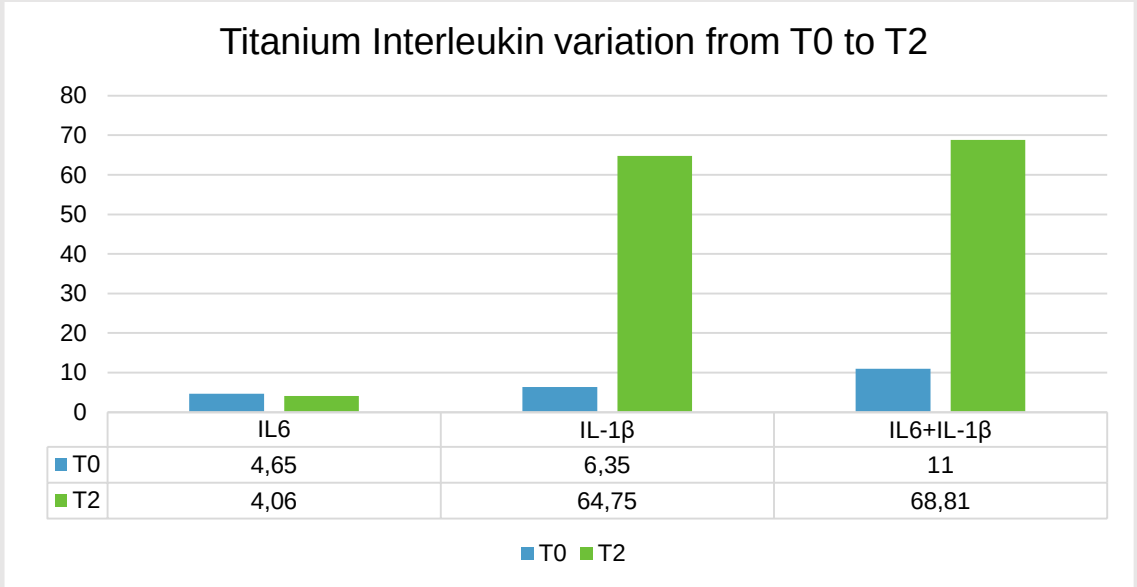


FIGURE 130 - Visual Display of Titanium Healing abutment (T) IL6, IL-1β and total interleukin variation between T0 (baseline) and T2 (eight weeks)

For titanium, only the non-parametric alternative for IL6 difference was used, with a p-value of 0.804 that is, the difference, in IL6, using titanium, was not significantly different to T0, so there were no differences between T0 and T2.

For IL-1β difference and total difference, the mean value was significantly dissimilar from 0 (using titanium) - *p*-values = 0.001. Given this positive difference, IL-1β at T2- IL-1β at T0> 0, so IL-1β was significantly higher at T2. Which was the same reasoning for total.

The statistical summary for interleukin variation on the titanium cad-cam abutment is shown in table 60.

Table 60 - Hypothesis and statistical conclusions of Titanium material with interleukins (IL-1β, IL6, Total) and different time frames (T0 and T2)

Interleukin	At T2 is H, L, E*	Test	Null Hypothesis	*P*-Value
IL6	S	Mann-Whitney	Retain	0,433
IL-1β	H	Mann-Whitney	Reject	0,00
IL6+ IL-1β	H	T-test	Reject	0,02

*H- the Interleukin concentration is Higher at T2 than at T0, L- the interleukin concentration is Lower at T2 than at T0, E the interleukin concentration is equal at T2 and at T0

CAD-Cam Acrylic Abutment and Interleukin variations

The Cad-Cam Acrylic (A) healing abutment was analyzed in the different IL concentrations to observe the pattern from T0 to T2.

The mean average and SD found are shown in table 61 and fig. 112

We can see once again that the pattern of the IL6 was similar to the titanium cad-cam abutment and there was only a small alteration of value from T0 to T2 but in this case a slight increase, not a slight decrease, as was seen in the titanium.

There was again, as in the titanium, a strong increase in the final average concentration values of IL-1β from T0 to T2, an increase that once again was reflected in the total inflammation value (IL6+IL-1β).

Although the variation from T0 to T2 was high, it did not exceed the higher variation found with the cad-cam titanium abutment.

Table 61 - Acrylic Healing Abutment (A) Mean Average and Standard Deviation of IL-1β, IL6 and total Interleukin through different time frame (T0 to T2)

	IL6	IL-1β	IL6+IL-1β
T0	7,63±6,58	5,31±3,16	12,95±7,78
T2	8,56±14,82	31,44±33,4	40±39,66

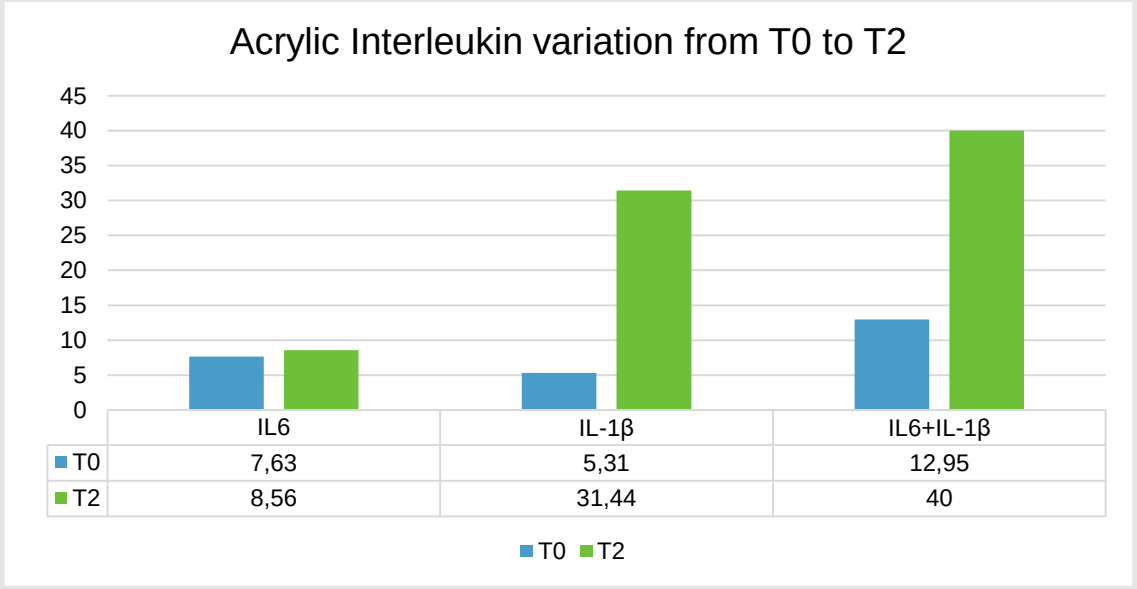


FIGURE 131 - Acrylic Healing abutment (A) IL6, IL-1β and total interleukin variation between T0 (baseline) and T2 (eight weeks)

The statistical conclusions were the same for the cad-cam titanium abutment (p-value of IL6 difference = 0,586; IL-1β difference = 0,000; total difference = 0,004), showingthat for IL6, the cad-cam Acrylic, was not significantly different than 0, with no differences between T0 and T2.

For IL-1β differences and total difference (IL-1β+IL6), the mean value was significantly different from 0 (using acrylic) *p*-values = 0,001. Given this positive

difference, IL-1β at T2- IL-1β at T0 > 0, so IL-1β was significantly higher at T2. The same reasoning stands for the combined interleukin.

The statistical conclusions were the same for the titanium abutment (*p*-value of IL6 difference = 0,586; IL-1β difference = 0,000; total difference = 0,004) and are presented in table 62.

Table 62 - Hypothesis and statistical conclusions of Acrylic material with interleukins (IL-1β, IL6, Total) and different time frames (T0 and T2)

Interleukin	At T2 is H, L, E*	Test	Null Hypothesis	*P*-Value
IL6	S	Mann-Whitney	Retain	0,22
IL-1β	H	Mann-Whitney	Reject	0,00
IL6+ IL-1β	H	Mann-Whitney	Reject	0,01

*H- the Interleukin concentration is Higher at T2 than at T0, L- the interleukin concentration is Lower at T2 than at T0, E the interleukin concentration is equal at T2 and at T0

CAD-Cam Zirconia abutment and interleukin variations

The Cad-Cam Zirconia (Z) healing abutment was analyzed in the different IL concentrations to observe the pattern from T0 to T2.

The mean average and SD found are shown in table 63 and fig. 113.

With regard to the IL, the concentration values were different from what the cad-cam acrylic and cad-cam titanium revealed. There was a decrease in concentration values from T0 to T2 for IL6, which contrasted to the increased IL6 concentration of the cad-cam acrylic abutment and the lack of concentration variation of the cad-cam titanium abutment.

Regarding IL-1β values, there was still an increase in value from T0 to T2 but the concentrations values at T2 were much lower that the values of the cad-cam acrylic and cad-cam titanium abutments.

These values influenced the total inflammation found at T2 which, although higher than the values in T0, were much lower than the same total values found for the cad-cam titanium and acrylic healing abutment.

Table 63 - Zirconia Healing Abutment (Z) Mean Average and Standard Deviation of IL-1β, IL6 and total Interleukin through different time frame (T0 to T2)

	IL6	IL-1β	IL6+IL-1β
T0	6,17±4,64	4,11±2,7	10,28±6,6
T2	4,76±13,83	29,94±54,07	34,7±55,99

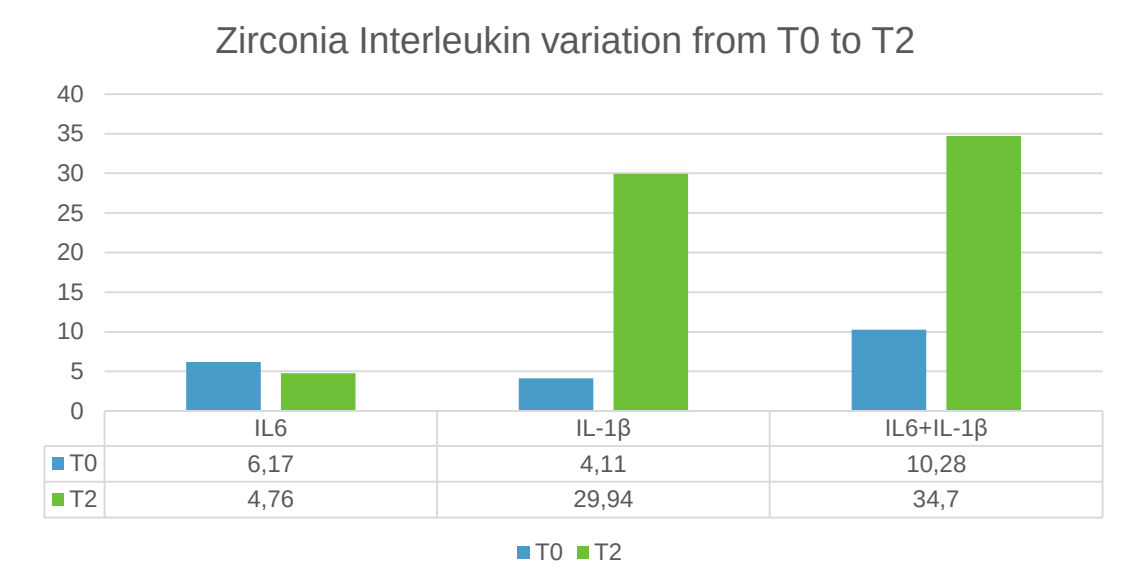


FIGURE 132 - Zirconia Healing abutment (A) IL6, IL-1β and total interleukin variation between T0 (baseline) and T2 (eight weeks)

In terms of final statistical values, the use of zirconia in all indicators, the mean difference was significantly dissimilar to 0 (*p*-values = 0,010, 0,000, and 0,010).

Therefore, using zirconia, IL-1β, and the total were significantly higher at T2, but for IL6 there were no changes (table 64). In fact, although not statistically different there was a decrease in value, as shown in table 63.

Table 64 - Hypothesis and statistical conclusions of Zirconia material with interleukins (IL-1β, IL6, Total) and different time frames (T0 and T2)

Interleukin	At T2 is H, L, E*	Test	Null Hypothesis	*P*-Value
IL6	S	Mann-Whitney	Retain	0,10
IL-1β	H	Mann-Whitney	Reject	0,00
IL6+IL-1β	H	Mann-Whitney	Reject	0,01

*H- the Interleukin concentration is Higher at T2 than at T0, L- the interleukin concentration is Lower at T2 than at T0, E the interleukin concentration is equal at T2 and at T0

Compare the interleukin differences found from T0 and T2, between the different materials (A, Z and T)

In this item we wanted to see if the differences found from T0 to T2 in each material, were different between materials**.**

The Kruskall-Wallis test was used to see if the differences varied with the materials. For all difference variables, *p*-values were higher than 0,05 (0,380, 0,090 and 0,093) as shown in table 65, and therefore did not differ significantly with the material.

In other words, the interleukin variations between time frame T0 to T2 were similar in the different biomaterials. (Z, A and T)

Table 65 - Hypothesis and statistical conclusions of the Interleukin difference found between T0 and T2 in each material (Z, T, A)

Interleukin Difference found between T0 and T2 in each material (Z, A, T)	Test	Null Hypothesis	*P*-Value
IL6 difference- Distribution across materials	kruskall-Wallis	Retain	0,380
IL-1β difference- Distribution across materials	kruskall-Wallis	Retain	0,09
IL6 +IL-1β difference- Distribution across materials	kruskall-Wallis	Retain	0,093

SECTION 5.2. PRIMARY OUTCOME MEASURES: INFLAMMATION LEVELS OF IL6 AND IL-1B ON IMPLANTS COMPARED TO INFLAMMATION LEVELS OF IL6 AND IL-1B IN PERIODONTAL CREVICULAR FLUID (PCF) - HYPOTHESIS AND RESULTS

Section 5.2.1. Hypothesis

FIGURE 133 - Titanium Healing abutment healed at T2.

Correlation Between Inflammation on peri-implant tissues of Implants Vs Inflammation on Periodontal Tissues of Teeth at T0 and T2 (8weeks) divided by Interleukin (IL6 and IL-1β)

This was undertaken to establish a correlation between peri-implant inflammation (independent of the abutment material used) and periodontal inflammation.

Specific aim 1: The correlation between the total amount of peri-implant Interleukins (IL6+IL-1β) and the total amount of periodontal Interleukins (IL6+ IL-1β) At T0 and T2 (8 Weeks), with the following assumptions:

H0: There is no difference in the Total amount of **peri-implant** Interleukins (IL6+ IL-1β), in implants placed under the standard protocol, compared to the Total amount of **periodontal** Interleukins (IL6+ IL-1β) at T0 and T2 (8 Weeks)

H1: There is a difference in the Total amount of **peri-implant** Interleukins (IL6+ IL-1β), in implants placed under the standard protocol, compared to the Total amount of **periodontal** Interleukins (IL6+ IL-1β) at T0 and T2 (8 Weeks)

Specific aim 2: The correlation between the total amount of peri-implant Interleukin (IL6) and the total amount of periodontal Interleukin (IL6) at T0 and T2 (8Weeks) with the following assumptions:

H0: There is no difference in the total amount of **peri-implant** Interleukins (IL6), in implants placed under the standard protocol, compared to the Total amount of **periodontal** Interleukin (IL6) at T0 and T2 (8 Weeks)

H1: There is a difference in the aotal amount of **peri-implant** Interleukins (IL6), in implants placed under the standard protocol, compared to the Total amount of **periodontal** Interleukin (IL6) at T0 and T2 (8 Weeks)

Specific aim 3: The correlation between total amount of peri-implant Interleukins (IL-1β) and total amount of periodontal Interleukin (IL-1β) at T0 and T2 (8 Weeks) with the following assumptions:

H0: There is no difference in the total amount of **peri-implant** Interleukins (IL-1β) in implants placed under the standard protocol, compared to the total amount of **periodontal** Interleukin (IL-1β) at T0 and T2 (8 Weeks)

H1: There is a difference in the total amount of **peri-implant** Interleukins (IL-1β), in implants placed under the standard protocol, compared to the total amount of **periodontal** Interleukin (IL-1β) at T0 and T2 (8 Weeks).

Correlation Between Inflammation on Implants Vs Inflammation on Teeth At T0 and T2 (8Weeks) By Biomaterial (Z, A, T)

This was undertaken to establish a correlation between peri-implant inflammation (total IL and IL6 and IL-1β) and periodontal inflammation (total IL and IL6 and IL-1β) based on the type of biomaterial used as an abutment (A vs Z vs T).

FOR CAD-CAM ZIRCONIA

Specific aim 4: To compare the total amount of peri-implant interleuikins (IL6+ IL-1β) based on the type of biomaterial - Zirconia, and the total amount of periodontal Interleukin (IL6+IL-1β) at T0 and T2 (8 Weeks) with the following assumptions:

H0: There is no difference in the **Total Amount of peri-implant Interleukins** (IL6+IL-1β) **on Zirconia** in implants placed under the standard protocol, compared to the total amount of **periodontal** Interleukin (IL6+IL-1β) at T0 and T2 (8 Weeks).

H1: There is a difference in the **Total Amount of peri-implant Interleukins** (IL6+ IL-1β) on Zirconia in implants placed under the standard protocol, compared to the total amount of **periodontal** Interleukin (IL6+IL-1β) at T0 and T2 (8 Weeks).

Specific aim 5: Based on the Type of Biomaterial the Total Amount of peri-implant Interleukins (IL6) and Total amount of periodontal Interleukin (IL6) on Zirconia at T0 and T2 (8 Weeks) with the following assumptions:

H0: There is no difference in the **Total Amount of peri-implant Interleukin** (IL6) on **Zirconia** in implants placed under the standard protocol, compared to the total amount of **periodontal** Interleukin (IL6) at T0 and T2 (8 Weeks)

H1: There is a difference in the **Total Amount of peri-implant Interleukins** (IL6) on **Zirconia** in implants placed under the standard protocol, compared to the total amount of **periodontal** Interleukin (IL6) at T0 and T2 (8 Weeks).

Specific aim 6; Based on the Type of Biomaterial Zirconia, Total Amount of peri-implant Interleukin (IL-1β) and Total amount of periodontal Interleukin (IL-1β) at T0 and T2 (8 Weeks) with the following assumptions:

H0: There is no difference in the **Total Amount of peri-implant Interleukin** (IL-1β) on **Zirconia** in implants placed under the standard protocol, compared to the Total amount of **periodontal** Interleukin (IL-1β) At T0 and T2 (8 Weeks)

H1: There is a difference in the **Total Amount of peri-implant Interleukins** (IL-1β) on **Zirconia** in implants placed under the standard protocol, compared to the Total amount of **periodontal** Interleukin (IL-1β) at T0 and T2 (8 Weeks).

FOR CAD-CAM TITANIUM

FIGURE 134 - CAD-CAM Titanium Healing abutment at T2 on the Maxilla

Specific aim 7: Based on the Type of Biomaterial, compare the Total Amount of peri-implant Interleukins (IL6+IL-1β) and the total amount of periodontal Interleukin (IL6+IL-1β) on Titanium at T0 and T2 (8 Weeks) with the following assumptions:

H0: There is no difference in the **Total Number of peri-implant Interleukins** (IL6+IL-1β) on Titanium in implants placed under the standard protocol, compared to the total amount of periodontal Interleukin (IL6+IL-1β) at T0 and T2 (8Weeks)

H1: There is a difference in the **Total Amount of peri-implant Interleukins** (IL6+ IL-1β) Titanium in implants placed under the standard protocol, compared to the Total amount of **periodontal** Interleukin (IL6+IL-1β) at T0 and T2 (8Weeks)

Specific aim 8: Based on the Type of Biomaterial Total Amount of peri-implant Interleukin (IL6) and the Total amount of periodontal Interleukin on Titanium (IL6) At T0 and T2 (8Weeks) with the following assumptions:

H0: There is no difference in the **Total Amount of peri-implant Interleukin** (IL6) on **Titanium** in implants placed under the standard protocol, compared to the Total amount of **periodontal** Interleukin (IL6) at T0 and T2 (8Weeks)

H1: There is a difference in the **Total Amount of peri-implant Interleukin** (IL6) on **Titanium** in implants placed under the standard protocol, compared to the Total amount of **periodontal** Interleukin (IL6) at T0 and T2 (8Weeks)

Specific aim 9: Based on the Type of Biomaterial compare the Total Amount of peri-implant Interleukin (IL-1β) and Total amount of periodontal Interleukin (IL-1β) on Titanium at T0 and T2 (8Weeks) with the following assumptions:

H0: There is no difference in the **Total Amount of peri-implant Interleukin** (IL-1β) on **Titanium** in implants placed under the standard protocol, compared to the total amount of **periodontal** Interleukin (IL-1β) at T0 and T2 (8Weeks)

H1: There is a difference in the **Total Amount of peri-implant Interleukin** (IL-1β) on **Titanium** in implants placed under the standard protocol, compared to the total amount of **periodontal** Interleukin (IL-1β) at T0 and T2 (8Weeks).

FOR CAD-CAM ACRYLIC

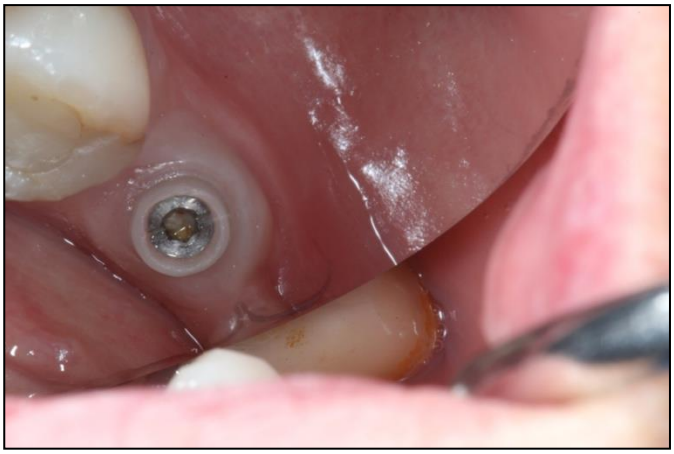


FIGURE 135 - CAD-CAM Acrylic abutment at T2

Specific aim 10: Based on the Type of Biomaterial, compare the Total Amount of peri-implant Interleukins (IL6+IL-1β) and the total amount of periodontal

Interleukins (IL6+IL-1β) on Acrylic at T0 and T2 (8 Weeks) with the following assumptions:

At T2 - 8Weeks

H0: There is no difference in the **Total Amount of peri-implant Interleukins** (IL6+IL-1β) on Acrylic in implants placed under the standard protocol, compared to the total amount of **periodontal** Interleukin (IL6+IL-1β) at T0 and T2 (8 Weeks).

H1: There is a difference in the **Total Amount of peri-implant Interleukins** (IL6+IL-1β) on **Acrylic** in implants placed under the standard protocol, compared to the Total amount of **periodontal** Interleukin (IL6+IL-1β) at T0 and T2 (8 Weeks).

Specific aim 11: Based on the Type of Biomaterial compare the Total Amount of peri-implant Interleukin (IL6) and the Total amount of periodontal Interleukin (IL6) on Acrylic at T0 and T2 (8 Weeks) with the following assumptions:

H0: There is no difference in the **Total Amount of peri-implant Interleukin** (IL6) on **Acrylic** in implants placed under the standard protocol, compared to the total amount of **periodontal** Interleukin (IL6) at T0 and T2 (8 Weeks)

H1: There is a difference in the **Total Amount of peri-implant Interleukin** (IL6) **on Acrylic** in implants placed under the standard protocol, compared to the total amount of **periodontal** Interleukin (IL6) at T0 and T2 (8 Weeks)

Specific aim 12: Based on the Type of Biomaterial compare the Total Amount of peri-implant Interleukin (IL-1β) and the Total amount of periodontal Interleukin (IL-1β) on Acrylic at T0 and T2 (8Weeks) with the following assumptions:

H0: There is no difference in the **Total Amount of peri-implant Interleukin** (IL-1β) on **Acrylic** in implants placed under the standard protocol, compared to the total amount of **periodontal** Interleukin (IL-1β) at T0 and T2 (8 Weeks)

H1: There is a difference in the **Total Amount of peri-implant Interleukin** (IL-1β) on **Acrylic** in implants placed under the standard protocol, compared to the total amount of **periodontal** Interleukin (IL-1β) at T0 and T2 (8 Weeks)

Section 5.2.2. Results

For the periodontal crevicular fluid (PCF) readings, the same extraction protocol was used for peri-implant crevicular fluid (PICF) collection.

The optical densities obtained were read by means of the same polynomial equation that was used on the PICF.

The results are shown in table 66.

Table 66 - IL-1β and IL6 table of Optical Densities (OD) and the corresponding concentrations values in pg/ml of periodontal crevicular fluids obtained from 20 Healthy subjects

pg/ml Tooth IL6	pg/ml Tooth IL-1β
-1	12
-4	7
-3	22
-1	22
-3	8
-2	8
-2	3
-1	25
-3	4
-3	3
-3	2
-2	54

0	33
-4	6
-1	13
-1	39
-3	6
-1	23
-1	6
-9	7

The PCF was analyzed in the different IL concentrations to observe the pattern at T2 (were the data for PCF was obtained) and at T0.

The mean average and SD found are shown in table 67 and fig. 117 and are contrasted with the PICF results.

Table 67 - Mean and standard deviation (SD) of concentrations in pg/ml of Interleukins at T0 Baseline and at T2, by Biomaterial and comparison with Periodontal Crevicular Fluid values (PCF) and Total Peri-implant Crevicular Fluid (PICF).

	PCF Total	PICF (total) T0	PICF (total) T2	PICF - A (T0)	PICF A (T2)	PICF Z (T0)	PICF Z (T2)	PICF T (T0)	PICF T (T2)
N for IL6	20	54	54	19	19	18	18	17	17
IL6	-2,4*	6,20 ± 5,43	6,02 ± 12,58	7,63 ±6,58	8,56 ±14,82	6,17 ±4,64	4,76 ±13,83	4,65 ±4,57	4,06 ±7,99
N for IL-1β	20	54	51	18	18	17	17	16	16
IL-1β	15,15	5,24 ± 3,91	41,39± 49,85	5,31 ±3,16	31,44 ±33,40	4,11 ± 2,7	29,94 ±54,07	6,35 ±5,37	64,75 ±55,24

*the value was adjusted to 0 since the biological meaning of 0 or minus 0 is the absence of IL6 in the fluid tested.

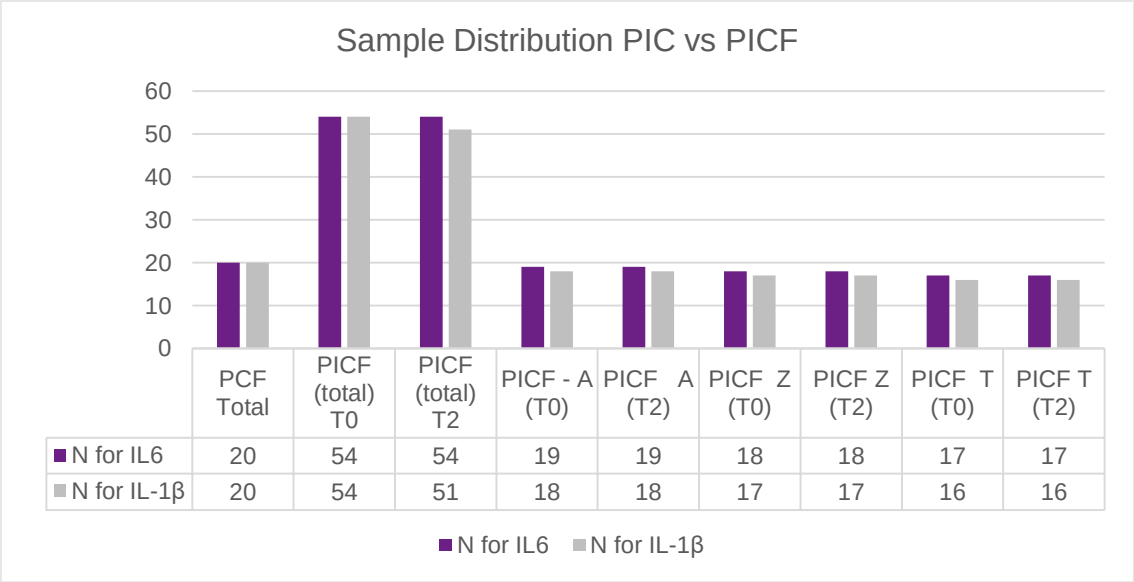


FIGURE 136 - N samples for different time frames of the laboratory procedures and statistical analysis. The results are different due to the presence of some IL-1β in the T2 samples which overshot the charted Elisa Interleukin concentration and thus did not provide an accurate result.

In an analysis the concentrations we can see that the presence of IL6 in the crevicular periodontal fluid was 0. In fact, the polynomial result from the OD was -2,4, but the biological significance was that, at the time the samples were drawn, there were no IL6 molecules present, as shown in table 118.

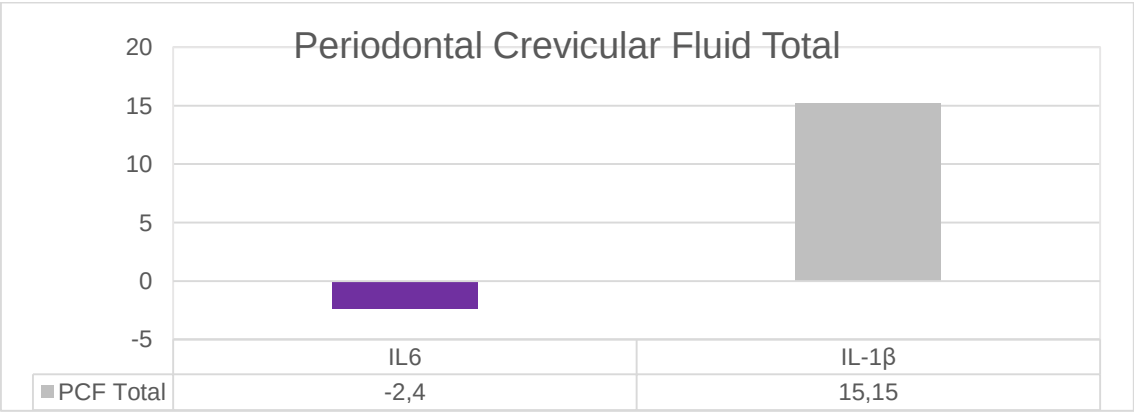


FIGURE 137 - Mean average of IL-1β and IL6 Interleukin concentrations for Periodontal crevicular fluid. Note the negative statistical result for IL6. This was adjusted to 0 since the biological translation of a negative result is the absence of interleukin in the samples read.

If we break down the comparison by material, the data was obtained as shown in fig. 119 and 120.

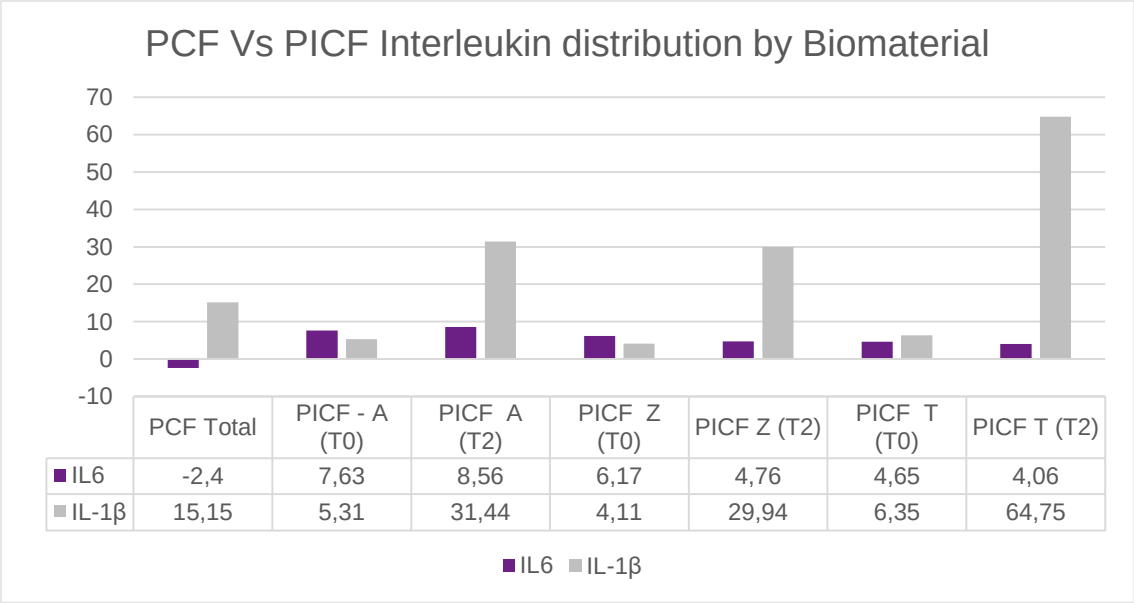


FIGURE 138 - Periodontal (PCF) and Peri-implant (PICF) Crevicular fluid concentration at different time frames (T0 and T2) by biomaterial (Z, A, T).

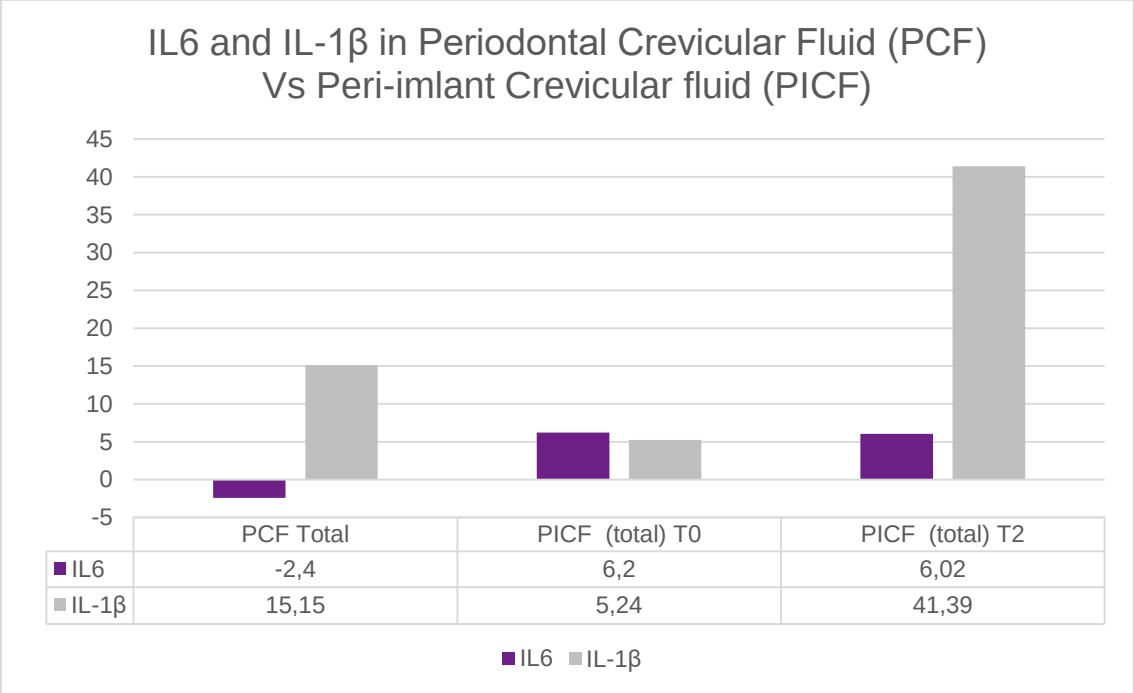


FIGURE 139 - Overall (independent of the material) Periodontal (PCF) and Peri-implant (PICF) Crevicular fluid concentration at different time frames (T0 and T2**)**

To compare PICF with PCF the mean of the PICF variable was used with a reference value for PCF. For this, the T test (large samples and / or normal distribution) was used when possible. When the T test was not possible due to sample size, then the Wilcoxon rank signed test was used.

When comparing interleukin 1β and 6, of PCF with PICF, two separate

moments were recorded: one comparison at T0 baseline, and another at T2 (8 Weeks).

In a separate comparison, the PICF was divided into different abutment biomaterial to see if there was an impact on inflammatory levels compared to PCF.

The mean reference values of a healthy periodontal sulcus in our study (n=20) was -2,4 pg/ml for IL6 (considered 0) and 15,15 pg/ml for IL-1β.

Total Concentration of PICF with total concentration of PCF independent of material

When comparing the total concentration of PICF of IL6 in all implants (54 readings) independent of the material (A, Z or T), we concluded that at the T0 baseline, the total IL6 (6,20 ± 5,43 pg/ml) was, on average, significantly higher than the value of the PCF of the tooth. (*p*=0,000) as shown in table 68.

At T2, IL6 of PICF (6,02 ± 12,58 pg/ml) was again, on average, significantly higher than the value of PCF (*p*=0,000).

This shows that at both time frames T0 and T2, with regard to IL6 independent of the material, there was statistically more inflammation expressed in the peri-implant tissues than on the periodontal tissues.

For the total PICF of IL-1β at T0, the mean value found was 5,24 ± 3,91, which was, on average, significantly lower than the value of the PCF (*p*=0,000) of the tooth. However, at T2, for PICF, IL-1β was found with a mean concentration of 41,39± 49,85 pg/ml which was, on average, significantly higher than the value of PCF. (*p*=0,00). This shows that at implant placement T0, inflammation was higher in periodontal tissues but once the peri-implant sulcus was established at T2 than the peri-implant site expressed more inflammation (IL-1β) than the healthy periodontal sulcus of adjacent teeth.

Table 68 - Hypothesis and statistical conclusions of the Overall Difference of IL6, IL-1β, between Periodontal Crevicular Fluid (PCF) and peri-implant crevicular fluid (PICF) At T0

PICF vs PCF	H, L, S*	Test	Null Hypothesis	*p*-Value
IL6 (overall implants) against PCF T0	H	One sample T-test	Reject	0,00
IL-1β (overall implants) against PCF T0	L	One sample T-test	Reject	0,00

*H- the Interleukin concentration is Higher at T2 than at T0, L- the interleukin concentration is Lower at T2 than at T0, E the interleukin concentration is equal at T2 and at T0

Table 69 - Hypothesis and statistical conclusions of the Overall Difference of IL6, IL-1β, between Periodontal Crevicular Fluid (PCF) and peri-implant crevicular fluid (PICF) At T2

PICF vs PCf	H, L, S*	Test	Null Hypothesis	P-Value
IL6 (overall implants) against PCF T2	H	One sample T-test	Reject	0,00
IL-1β (overall implants) against PCF T2	H	One sample T-test	Reject	0,00

*H- the Interleukin concentration is Higher at T2 than at T0, L- the interleukin concentration is Lower at T2 than at T0, E the interleukin concentration is equal at T2 and at T0

To check if the healing abutment material had an impact on the levels of PICF compared to PCF the immune response was broken up into three different types.

For Cad-cam Titanium PICF vs PCF

For cad-cam titanium (T), two measures were recorded, one at T0 that showed that PICF of IL6 had a mean of 4,65 ± 4,57 pg/ml, and was, on average,

significantly higher than the value of the PCF (*p*=0,00). This was the same at T2 (4,06 ± 7,99 pg/ml) where IL6 was again, on average, significantly higher than the value of the tooth. (*p*=0,015)

At T0, for titanium, the IL-1β, 6,35 ± 5,37 pg/ml was, on average, significantly, lower than the value of the periodontal tissues of teeth. (*p*=0,00)

At T2, for titanium, IL-1β mean 64,75 ± 55,24 pg/ml was, on average, significantly higher than the value of the tooth. (*p*=0,03). As shown in table 70, the behavior of IL-1β is different to the behavior of IL6 for the titanium healing abutment. IL6 on PICF, both at T0 or at T2, was always higher than the PCF, showing that Implant/abutment placement triggered a response from day 0 throughout the osseointegration process (table 70).

Table 70 - Hypothesis and statistical conclusions of Titanium Difference in IL6, IL-1β and Total IL, between Periodontal Crevicular Fluid (PCF) and peri-implant crevicular fluid (PICF) At T0 and T2

PICF vs PCf	H, L, S*	Test	Null Hypothesis	P-Value
IL6 (overall implants) against PCF at T0	H	One sample T-test	Reject	0,00
IL-1β (overall implants) against PCF at T0	L	One sample T-test	Reject	0,00
IL6 (overall implants) against PCF at T2	H	One sample T-test	Reject	0,001
IL-1β (overall implants) against PCF at T2	H	One sample T-test	Reject	0,001

For Cad-cam Acrylic PICF vs PCF

For the cad-cam Acrylic Healing abutment, the results were:

At T0, IL6 (7,63 ± 6,58 pg/ml) from the PICF was, on average, significantly higher than the value of the PCF (*p*=0,00), which were same for IL6 (8,56 ±

14,82 pg/ml) at T2 where, on average, they were significantly higher than the value of the PCF. (*p*=0,00)

At T0, IL-1β had a mean value of 5,31 ± 3,16 pg/ml which was, on average, significantly lower than the value of the PCF. (*p*=0,000)

At T2, for the same interleukin IL-1β (31,44 ± 33,40 pg/ml), the results were not significantly different from the value of the PCF (*p*=0,058). (Table 71)

Table 71 - Hypothesis and statistical conclusions of Acrylic Difference in IL6, IL-1β and Total IL, between Periodontal Crevicular Fluid (PCF) and peri-implant crevicular fluid (PICF) At T0 and T2

PICF vs PCf	H, L, S*	Test	Null Hypothesis	P-Value
IL6 (overall implants) against PCF at T0	H	One sample T-test	Reject	0,00
IL-1β (overall implants) against PCF at T0	L	One sample T-test	Reject	0,00
IL6 (overall implants) against PCF at T2	H	One sample T-test	Reject	0,001
IL-1β (overall implants) against PCF at T2	S	One sample T-test	Retain	0,058

*H- the Interleukin concentration is Higher at T2 than at T0, L- the interleukin concentration is Lower at T2 than at T0, E the interleukin concentration is equal at T2 and at T0

For Cad-cam Zirconia PICF vs PCF

For the Zirconia Group Healing abutment, the results were:

At T0, the mean value for the IL6 was 6,17 ± 4,64 pg/ml which was, on average, significantly higher than the value of the periodontal fluid of the adjacent teeth.

At T2, the same IL6 had a mean value of 4,76 ± 13,83 pg/ml and was, on average similar than the value of the tooth. (*p*=0,560)

Herein lies a big difference between zirconia abutment and the titanium and

acrylic abutment. For T and A, IL6 was always higher in PICF than PCF in both time frames T0 and T2, but in the cad-cam zirconia group at T0, PICF was similar to that of the PCF of adjacent teeth, which in theory means less inflammation at implant placement.

At T0, for IL-1β the mean value found was 4,11 ± 2,7 pg/ml which was, on average, significantly lower than the value for the tooth. (*p*=0,000)

At T2, the same IL-1β had an average value of 29,94 ± 54,07 pg/ml and was not significantly different from the value for the tooth (*p*=0,943). (Table 72)

IL-1β acrylic and zirconia revealed different behavior when we compared PICF at T2 with PCF. For these two abutments the behavior was the same with no statistical differences between them. But when we compare them to cad-cam titanium we see that for this abutment at T2 for IL-1β PICF expresses a higher mean value than the PCF. This means that at T2 the titanium only has more inflammation than the PCF for the tooth, the A and Z abutment had an inflammatory response equal to the PCF of adjacent teeth.

Table 72 - Hypothesis and statistical conclusions of Zirconia Difference of IL6, IL-1β and Total IL, between Periodontal Crevicular Fluid (PCF) and peri-implant crevicular fluid (PICF) At T0 and T2

PICF vs PCf	H, L, S*	Test	Null Hypothesis	P-Value
IL6 (overall implants) against PCF at T0	H	One sample T-test	Reject	0,00
IL-1β (overall implants) against PCF at T0	L	One sample T-test	Reject	0,00
IL6 (overall implants) against PCF at T2	S	One sample T-test	Retain	0,56
IL-1β (overall implants) against PCF at T2	S	One sample T-test	Retain	0,943

*H- the Interleukin concentration is Higher at T2 than at T0, L- the interleukin concentration is Lower at T2 than at T0, E the interleukin concentration is equal at T2 and at T0

SECTION 5.3. PRIMARY OUTCOME MEASURES: INFLAMMATION LEVELS OF IL6 AND IL-1β COMPARED TO INFLAMMATION LEVELS OF IL6 AND IL-1β IN BLOOD SAMPLES (BF) AT BASELINE (T0)- HYPOTHESIS AND RESULTS

Section 5.3.1. Hypothesis

To establish a correlation between peri-implant inflammation (independent of the abutment material used) and blood inflammation (BF), by Interleukin.

Specific aim 1: Correlation Between total amount of peri-implant Interleukins (IL6+ IL-1β) and the total amount of blood Interleukins (IL6+IL-1β) At T0 – Baseline

H0: There is no difference in the total amount of peri-implant Interleukins **(IL6+ IL-1β)** on implants placed under the standard protocol **At T0 - Baseline**, compared to the total amount of **blood Interleukin (IL6+ IL-1β)**

H1: There is a difference in the total amount of peri-implant Interleukins **(IL6+ IL-1β)** on implants placed under the standard protocol **At T0 - Baseline**, compared to the Total amount of **blood Interleukin (IL6+ IL-1β)**

Specific aim 2: To establish a correlation between total amount of peri-implant Interleukin (IL6) and total amount of blood Interleukin (IL6) at T0- Baseline

H0: There is no difference in the total amount of peri-implant Interleukin **(IL6)** on implants placed under the standard protocol **At T0 - Baseline**, compared to the total amount of **blood Interleukin (IL6).**

H1: There is a difference in the total amount of peri-implant Interleukin **(IL6)** on implants placed under the standard protocol **At T0 - Baseline**, compared to the Total amount of **blood Interleukin (IL6)**

Specific aim 3: To establish a correlation between total amount of peri-implant Interleukin (IL-1β) and total amount of blood Interleukin (IL-1β) at T0 – Baseline

H0: There is no difference in the total amount of peri-implant Interleukin (IL-1β) on implants placed under the standard protocol **at T0 - Baseline**, compared to the total amount of blood Interleukin (IL-1β)

H1: There is a difference in the total amount of peri-implant Interleukin (IL-1β) on implants placed under the standard protocol **At T0 - Baseline**, compared to the total amount of blood Interleukin (IL-1β).

Correlation Between Inflammation on Implants Vs Blood Inflammation at T0 (baseline): To establish a correlation between peri-implant inflammation (total IL and IL6 and IL-1β) and blood inflammation (total IL and IL6 and IL-1β) **based on the type of biomaterial** used as an abutment (A vs Z vs T).

FOR CAD-CAM ZIRCONIA

Specific aim 4: To compare the total amount of peri-implant Interleukins (IL6+ IL-1β) and the total amount of blood Interleukins (IL6+IL-1β) based on the Type of Biomaterial in zirconia, at T0 – Baseline.

H0: There is no difference in the **total amount of peri-implant Interleukins** (IL6+ IL-1β) on Zirconia in implants placed under the standard protocol **at T0 - Baseline**, compared to the total amount of blood Interleukin (IL6+IL-1β).

H1: There is a difference in the **total amount of peri-implant Interleukins** (IL6+ IL-1β) on **Zirconia** in implants placed under the standard protocol **At T0 - Baseline**, compared to the total amount of blood Interleukin (IL6+ IL-1β).

Specific aim 5: To compare the total amount of peri-implant Interleukin (IL6) and total amount of blood Interleukin (IL6) at T0 – baseline, based on the Type of Biomaterial on Zirconia.

H0: There is no difference in the **total amount of peri-implant Interleukin** (IL6) on **Zirconia** in implants placed under the standard protocol **at T0 - Baseline**, compared to the total amount of blood Interleukin (IL6).

H1: There is a difference in the **total amount of peri-implant Interleukin** (IL6) on **Zirconia** in implants placed under the standard protocol **At T0 - Baseline**, compared to the total amount of blood Interleukin (IL6).

Specific aim 6: To compare the total amount of peri-implant Interleukin (IL-1β) and the total amount of blood Interleukin (IL-1β) based on the type of biomaterial, at T0 – baseline on Zirconia.

H0: There is no difference in the **total amount of peri-implant Interleukin** (IL-1β) on **Zirconia** in implants placed under the standard protocol **at T0 - Baseline**, compared to the total amount of blood Interleukin (IL-1β).

H1: There is a difference in the **total amount of peri-implant Interleukin** (IL-1β) on **Zirconia** in implants placed under the standard protocol **at T0 - Baseline**, compared to the total amount of blood Interleukin (IL-1β).

FOR CAD-CAM TITANIUM

Specific aim 7: To compare the total amount of peri-implant Interleukins (IL6+ IL-1β) and total amount of blood Interleukins (IL6+IL-1β) based on the Type of Biomaterial on Titanium.

At T0 - Baseline

H0: There is no difference in the **total amount of peri-implant Interleukins** (IL6+ IL-1β) on t**itanium** in implants placed under the standard protocol **at T0 - Baseline**, compared to the total amount of blood Interleukin (IL6+IL-1β).

H1: There is a difference in the **total amount of peri-implant Interleukins** (IL6+ IL-1β) **on titanium** in implants placed under the standard protocol **at T0 - Baseline**, compared to the total amount of blood Interleukins (IL6+IL-1β).

Specific aim 8: To compare the total amount of peri-implant Interleukin (IL6) and total amount of blood Interleukin (IL6) based on the Type of Biomaterial on titanium, at T0 – Baseline.

H0: There is no difference on the **Titanium, when** comparing the **total amount of peri-implant Interleukin** (IL6) in implants placed under the standard protocol **at T0 - Baseline** to the Total amount of blood Interleukin (IL6)

H1: There is a difference in the **total amount of peri-implant Interleukin** (IL6) on **Titanium** in implants placed under the standard protocol **at T0 - Baseline**, compared to the total amount of blood Interleukin (IL6).

Specific aim 9: To compare the total amount of peri-implant Interleukin (IL-1β) and the total amount of blood Interleukin (IL-1β) based on the type of biomaterial on titanium, at T0 – Baseline.

H0: There is no difference in the **total amount of peri-implant Interleukin** (IL-1β) on t**itanium** in implants placed under the standard protocol **at T0 - Baseline**, compared to the total amount of blood Interleukin (IL-1β).

H1: There is a difference in the **total amount of peri-implant Interleukins** (IL-1β) on **Titanium** in implants placed under the standard protocol **at T0 - Baseline**, compared to the total amount of blood Interleukin (IL-1β).

FOR CAD-CAM ACRYLIC

Specific aim 10: To compare the total amount of peri-implant Interleukins (IL6+ IL-1β) and the total amount of blood Interleukins (IL6+ IL-1β), based on the type of biomaterial on Acrylic, at T0 – Baseline.

H0: There is no difference in the **total amount of peri-implant Interleukins** (IL6+ IL-1β) on **Acrylic** in implants placed under the standard protocol **at T0 - Baseline**, compared to the total amount of blood Interleukins (IL6+IL-1β).

H1: There is a difference in the **total amount of peri-implant Interleukins** (IL6+ IL-1β) on **Acrylic** in implants placed under the standard protocol **at T0 - Baseline**, compared to the total amount of blood Interleukin (IL6+IL-1β).

Specific aim 11: To compare the total amount of peri-implant Interleukin (IL6) and total amount of blood Interleukin (IL6) based on the Type of Biomaterial on acrylic at T0 – Baseline.

H0: There is no difference in the **total amount of peri-implant Interleukin** (IL6) on **Acrylic** in implants placed under the standard protocol **at T0 - Baseline**, compared to the Total amount of blood Interleukin (IL6).

H1: There is a difference in the **total amount of peri-implant Interleukin** on **Acrylic** (IL6) in implants placed under the standard protocol **at T0 - Baseline**, compared to the total amount of blood Interleukin (IL6).

Specific aim 12: To compare the total amount of per implant Interleukin (IL-1β) and the total amount of blood Interleukin (IL-1β) based on the Type of Biomaterial on Acrylic At T0 – Baseline.

H0: There is no difference in the **Total Amount of peri-implant Interleukin** (IL-1β) on Acrylic in implants placed under the standard protocol **at T0 - Baseline**, compared to the total amount of blood Interleukin (IL-1β).

H1: There is a difference in the **total amount of peri-implant Interleukin** (IL-1β) on **Acrylic** in implants placed under the standard protocol **at T0 - Baseline**, compared to the total amount of blood Interleukin (IL-1β).

Section 5.3.2. Results

For the Blood Fluid (BF) analysis, a similar extraction protocol was undertaken to the periodontal crevicular fluid (PCF) and the peri-implant crevicular fluid (PICF).

Readings were recorded by means of the same protocol than the one used for the collection of peri-implant crevicular fluid (PICF).

The optical densities obtained were read under the same polynomial equation.

The results are shown in table 73.

Table 73 - IL-1β and IL6 General Summary of Optical Densities (OD) obtained and the corresponding concentrations values in pg/ml of blood fluids (BF), in 12 Healthy subjects

Pg/dl IL6 Blood	Pg/dl IL-1β Blood
-1	1
-1	2
-1	1
0	1
-1	6
1	2
-1	3
0	1
-1	30
-1	2
-2	1
-1	1

The mean average and standard deviation of PICF and PCF were measured and compared to the mean obtained for Blood Fluid (BF) as shown in Table 74 and fig. 115.

The mean value found for the Bf was -0.75 pg/ml for IL6, once again just as in the values found for IL6 on PCF, the biological significance was that there was no IL available, so 0 and 4.25 pg/ml for IL-1β. was considered.

Table 74 - Mean and standard deviation of concentration in pg/ml of Interleukins at T0 Baseline and at T2 by Biomaterial and the comparison with Periodontal Crevicular Fluid values (PCF) Total Peri-implant Crevicular Fluid (PICF) and Blood Fluid (BF).

	BF Total	PCF Total	PICF (total) T0	PICF (total) T2	PICF A (T0)	PICF A (T2)	PICF Z (T0)	PICF Z (T2)	PICF T (T0)	PICF T (T2)
N for IL6	12	20	54	54	19	19	18	18	17	17
IL6	-0,75*	-2,4	6,20 ±5,43	6,02 ± 12,58	7,63 ±6,58	8,56 ±14,82	6,17 ±4,64	4,76 ±13,83	4,65 ±4,57	4,06 ±7,99
N for IL-1β	12	20	54	51	18	18	17	17	16	16
IL-1β	4,25	15,15	5,24 ±3,91	41,39 ±49,85	5,31 ±3,16	31,44 ±33,40	4,11 ±2,7	29,94 ±54,07	6,35 ±5,37	64,75 ±55,24

*the value obtained was adjusted to 0 since the biological meaning of 0 or minus 0 is the absence of IL6 in the fluid tested

In fig. 121 is shown the sample distribution for the 3 types of biological fluids taken from the PICF, the PCF and the BF.

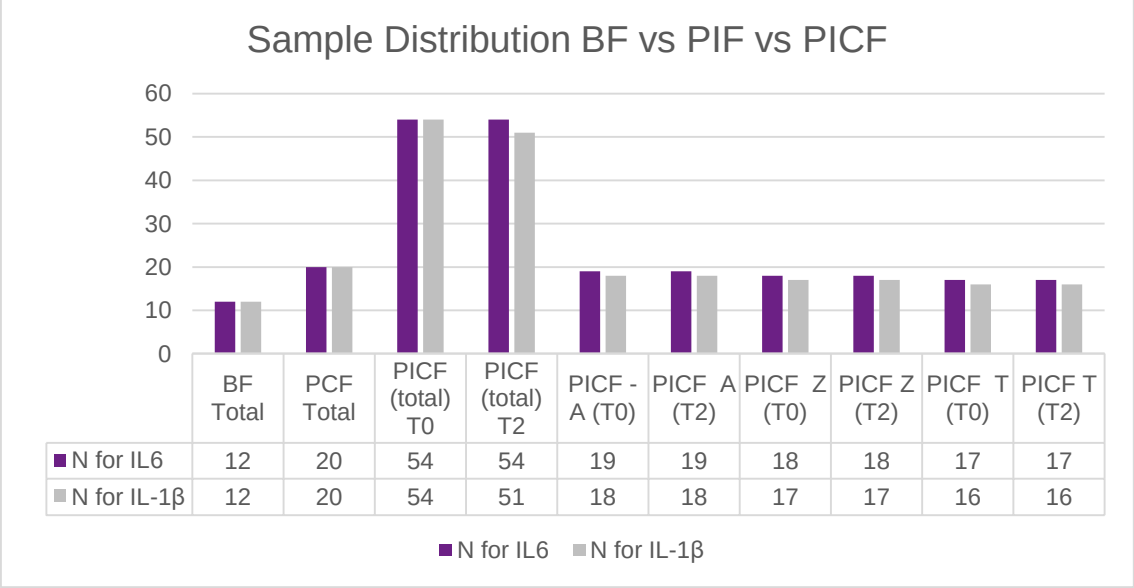


FIGURE 140 - Peri-implant Crevicular Fluid (PICF), Periodontal Crevicular Fluid (PCF) and Blood Fluid (BF) samples for different time frames of the laboratory procedures and statistical analysis. The results are different due to some IL-1β at T2 samples overcharting the Elisa Interleukin concentration and thus not giving an accurate result.

Interleukin was first analyzed at different time frames (T0 and T2):

Comparing BF with the total amount of IL at T0 and T2 independent of the material provided another control group.

IL6 results showed an obvious difference between BF and PICF either at T0 and at T2, leading us to conclude, at T0, IL6 was, on average, significantly higher than the blood value (p=0.00) in those time frames

IL-1β revealed different results where the reference values were compared to PICF and at T0 there were no differences in the expression of IL-1β, but at T2 the difference was greater. The final statistics methods showed that, at T0, IL-1β was not, on average, significantly different from the blood value. (p=0.068) but at T2, IL-1β concentrations were very different, being on average higher at T2 in the PICF.

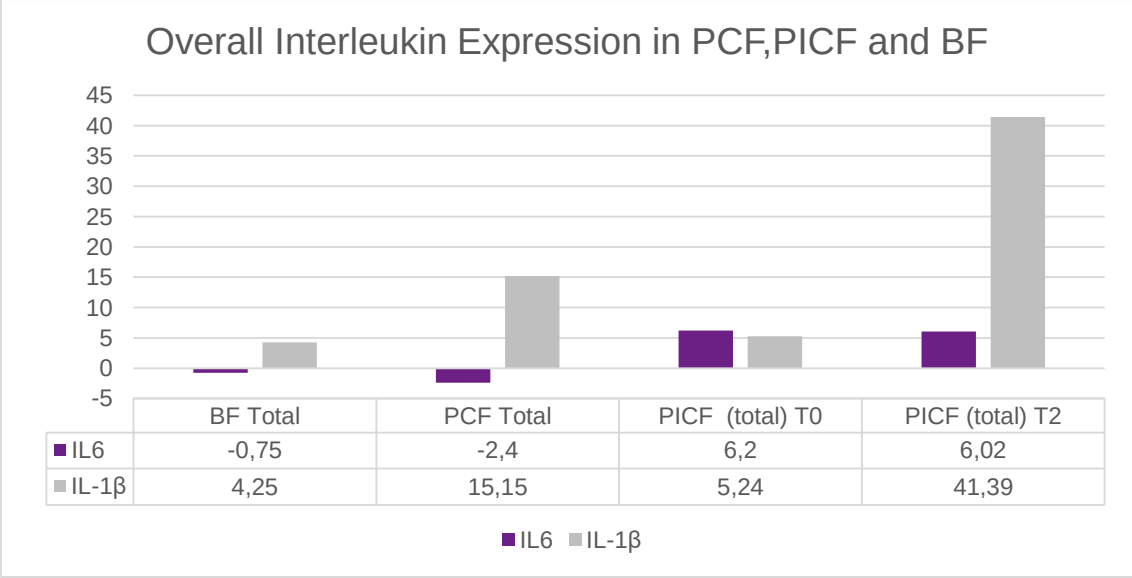
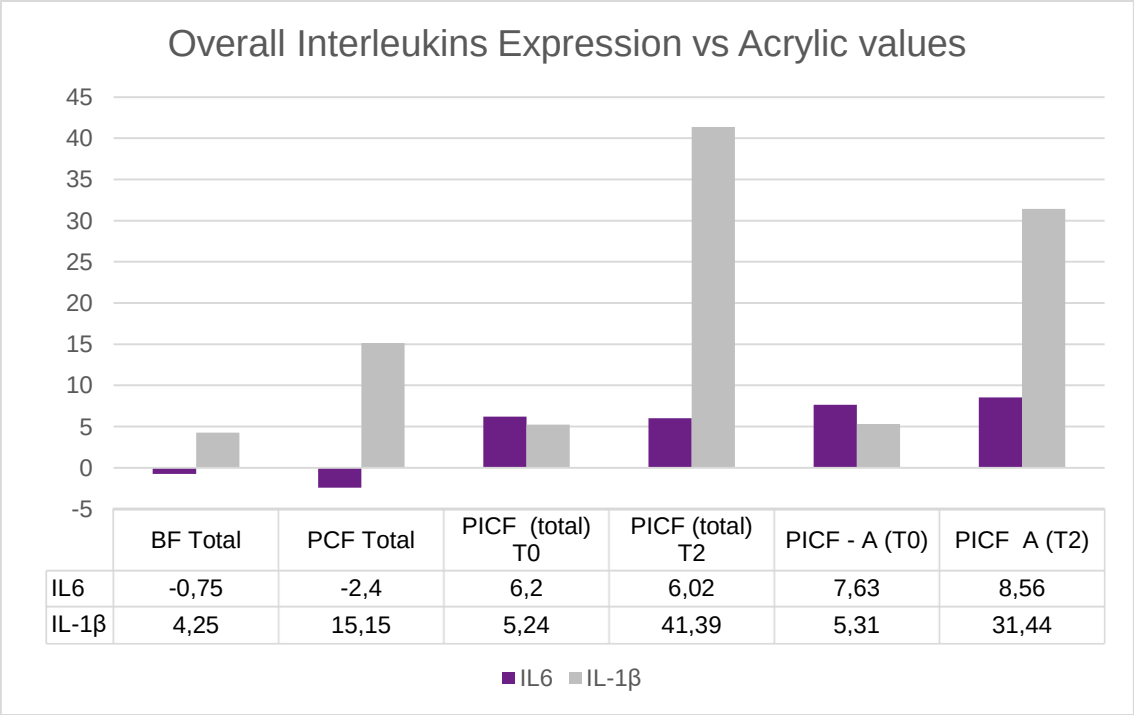


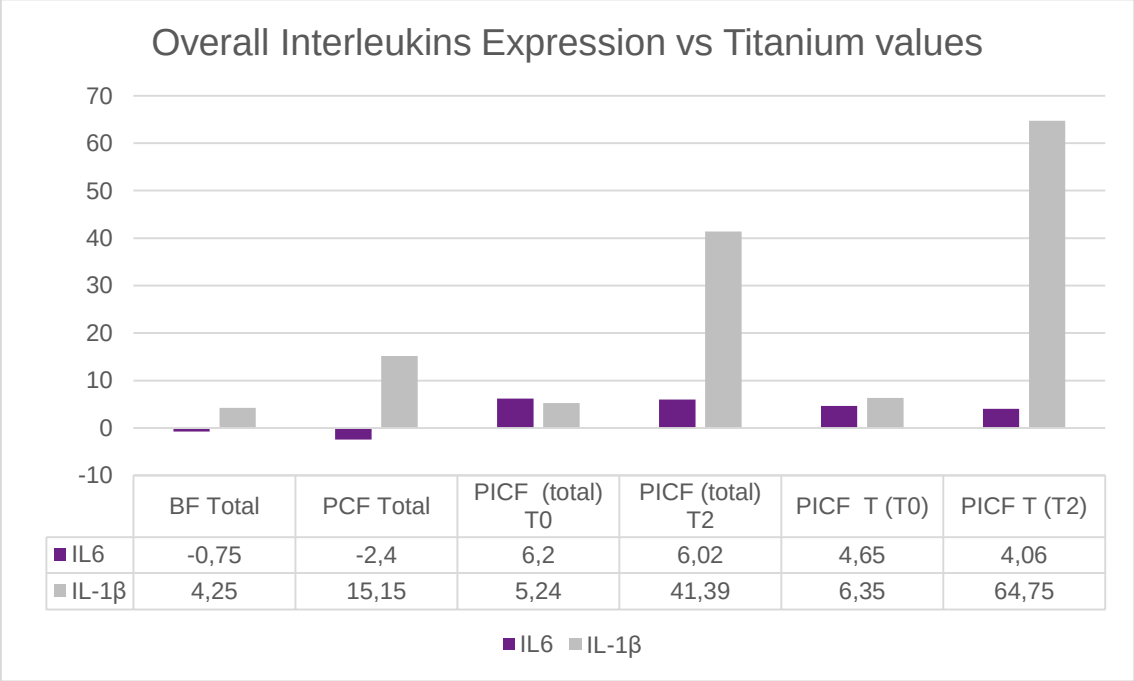
FIGURE 141 - Overall Peri-implant Crevicular Fluid (PICF), Periodontal Crevicular Fluid (PCF) and Blood Fluid (BF) interleukin Expression. Note that the -0,75 IL6 result was adjusted to 0 for statistical comparison

If we break down the comparison by material, we obtained data as shown in fig. 123.



	BF Total	PCF Total	PICF (total) T0	PICF (total) T2	PICF - A (T0)	PICF A (T2)
IL6	-0,75	-2,4	6,2	6,02	7,63	8,56
IL-1β	4,25	15,15	5,24	41,39	5,31	31,44

FIGURE 142 - Overall Peri-implant Crevicular Fluid (PICF), Periodontal Crevicular Fluid (PCF) and Blood Fluid (BF) interleukin Expression and comparison with Acrylic Interleukin expression at different time frames. Note that the -0,75 IL6 result was adjusted to 0 for statistical comparison



	BF Total	PCF Total	PICF (total) T0	PICF (total) T2	PICF T (T0)	PICF T (T2)
IL6	-0,75	-2,4	6,2	6,02	4,65	4,06
IL-1β	4,25	15,15	5,24	41,39	6,35	64,75

FIGURE 143 - Overall Peri-implant Crevicular Fluid (PICF), Periodontal Crevicular Fluid (PCF) and Blood Fluid (BF) interleukin Expression and comparison with Titanium Interleukin expression at different time frames.

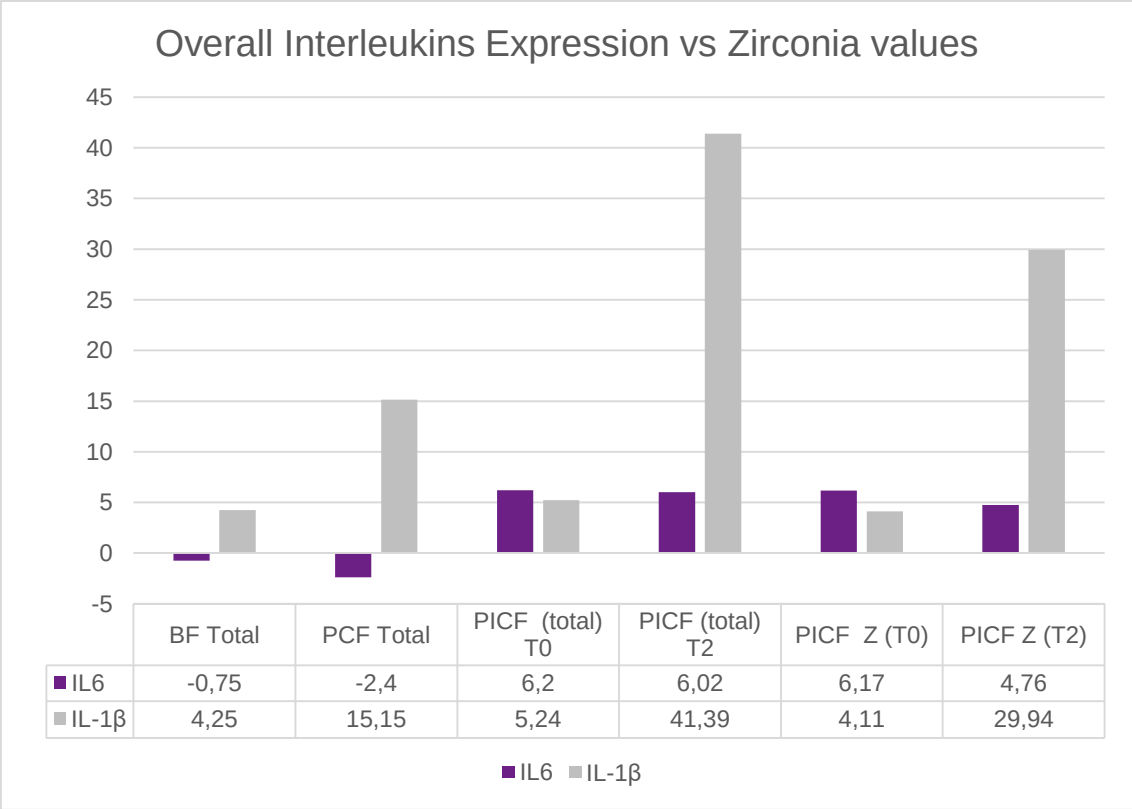


FIGURE 144 - Overall Peri-implant Crevicular Fluid (PICF), Periodontal Crevicular Fluid (PCF) and Blood Fluid (BF) interleukin Expression and comparison with Zirconia Interleukin expression at different time frames. Note that the -0,75 IL6 result was adjusted to 0 for statistical comparison

Comparison of Blood Fluids vs PICF (by time frame) at T0

With regard to IL6, All T0 values for the different biomaterials were higher than the blood fluid values, since there was an absence of IL6 in BF at the time of incision (fig. 123,124 and 125).

Thus, there were statistically significant values between those items.

In summary, (74) the statistical methodology showed us that at T0, for acrylic IL6 was, on average, significantly higher than the blood value (p=0,00) **and** for zirconia, IL6 was, on average, significantly higher than the blood value (p=0,00).

At T0, for Titanium, IL6 was, on average, significantly higher than the blood value (p=0,00) (table 77,78,79).

With regard to IL-1β the basal blood value found at T0 was 4,25 pg/ml. so when we broke it down by material, at T0, titanium (6,35 pg/ml), was not significantly different from the blood value (p=0,377).

At T0, for acrylic (5,31), IL-1β was not significantly different from the blood value (*p*= 0,133) and at T0, for zirconia (4,11), IL-1β was not significantly different from the blood value (*p*= 0,710) (table 77,78,79).

Comparison of Blood Fluids vs PICF (by time frame) at T2

At T2 for IL6 the behavior was similar than at T0, mainly because at T2 the IL6 concentration was again much higher than the VF at the time of incision.

Generally, independent of the abutment material, at T2, IL6 and IL-1β were, on average, significantly higher than the blood value (*p*=0,00).

When we brake it down by material then at T2, for titanium, IL6 was, on average, significantly higher than the blood value. (*p*= 0,15), and also at T2, for the same healing abutment, IL-1β was, on average, significantly higher than the blood value. (*p*= 0,01)

At T2, for acrylic, IL6 was, on average, significantly higher than the blood value. (*p*= 0,00) and same for the IL-1β which was, on average, significantly higher than the blood value (*p*= 0.00).

At T2, for zirconia, IL-1β was, on average, significantly higher than the blood value. (*p*=0,01) and at T2, for zirconia, IL6 was, on average, significantly higher than the blood value (*p*= 0,09).

Table 75 - Hypothesis and statistical conclusions of the Overall Difference of IL6, IL-1β, between Blood Fluid (BF) and peri-implant crevicular fluid (PICF) At T0

PICF vs BF	H, L, S*	Test	Null Hypothesis	P-Value
IL6 (overall implants) against BF T0	H	One sample T-test	Reject	0,00
IL-1β (overall implants) against BF T0	S	One sample T-test	Retain	0,068

*H- the Interleukin concentration is Higher at T2 than at T0, L- the interleukin concentration is Lower at T2 than at T0, E the interleukin concentration is equal at T2 and at T0

Table 76 - Hypothesis and statistical conclusions of the Overall Difference of IL6, IL-1β, between Blood Fluid (BF) and peri-implant crevicular fluid (PICF) At T2

PICF vs BF	H, L, S*	Test	Null Hypothesis	*P*-Value
IL6 (overall implants) against BF T2	H	One sample T-test	Reject	0,00
IL-1β (overall implants) against BF T2	H	One sample T-test	Reject	0,00

*H- the Interleukin concentration is Higher at T2 than at T0, L- the interleukin concentration is Lower at T2 than at T0, E the interleukin concentration is equal at T2 and at T0

Table 77 - Hypothesis and statistical conclusions of ifference of IL6 in Titanium, IL-1β and Total IL, between Blood Fluid (BF) and peri-implant crevicular fluid (PICF) At T0 and T2

PICF vs BF	H, L, S*	Test	Null Hypothesis	*P*-Value
IL6 (overall implants) against BF at T0	H	One sample T-test	Reject	0,00
IL-1β (overall implants) against BF at T0	S	One sample T-test	Retain	0,377
IL6 (overall implants) against BF at T2	H	One sample T-test	Reject	0,015
IL-1β (overall implants) against BF at T2	H	One sample T-test	Reject	0,001

*H- the Interleukin concentration is Higher at T2 than at T0, L- the interleukin concentration is Lower at T2 than at T0, E the interleukin concentration is equal at T2 and at T0

Table 78 - Hypothesis and statistical conclusions of Acrylic Difference of IL6, IL-1β and Total IL, between Blood Fluid (BF) and peri-implant crevicular fluid (PICF) At T0 and T2.

PICF vs BF	H, L, S*	Test	Null Hypothesis	*P*-Value
IL6 (overall implants) against BF at T0	H	One sample T-test	Reject	0,00
IL-1β (overall implants) against BF at T0	S	One sample T-test	Retain	0,133
IL6 (overall implants) against BF at T2	H	One sample T-test	Reject	0,001
IL-1β (overall implants) against BF at T2	H	One sample T-test	Reject	0,000

*H- the Interleukin concentration is Higher at T2 than at T0, L- the interleukin concentration is Lower at T2 than at T0, E the interleukin concentration is equal at T2 and at T0

Table 79 - Hypothesis and statistical conclusions of Zirconia Difference of IL6, IL-1β and Total IL, between Blood Fluid (BF)) and peri-implant crevicular fluid (PICF) At T0 and T2

PICF vs BF	H, L, S*	Test	Null Hypothesis	*P*-Value
IL6 (overall implants) against BF at T0	H	One sample T-test	Reject	0,00
IL-1β (overall implants) against BF at T0	S	One sample T-test	Retain	0,710
IL6 (overall implants) against BF at T2	H	One sample T-test	Reject	0,009
IL-1β (overall implants) against BF at T2	H	One sample T-test	Reject	0,001

*H- the Interleukin concentration is Higher at T2 than at T0, L- the interleukin concentration is Lower at T2 than at T0, E the interleukin concentration is equal at T2 and at T0

SECTION 5.4. PRIMARY OUTCOME MEASURES: ZIRCONIA, ACRYLIC AND TITANIUM INFLAMMATION LEVELS OF IL6 AND IL-1β AND CORRELATION TO MARGINAL BONE LOSS (MBL) - HYPOTHESIS AND RESULTS

Section 5.4.1. Hypothesis

Correlation Between Inflammation Vs Abutment Material (Z, T, A) Vs Marginal Bone Loss

The aim was to correlate the amount of marginal bone resorption (MBL) with the concentrations of interleukins present at T2, depending on the abutment material used.

When studying marginal bone loss, we use two types of measures or to be more specific two different patterns of marginal bone remodeling (measure A and B, also known as MBL1 MBL2, respectively) and compare inflammation levels with these two types of measurements/radiological features.

On the day of surgery all implants were all placed 2 mm below the crestal bone, which means that at baseline (T0), marginal bone always remained coronal to the implant platform.

After 8 weeks two scenarios were evident: 1- in some implants bone remaineded coronal to the implant platform with bone loss or 2- in some implants there was bone resorption that went apical to the implant platform.

In some implants scenario 1 occurred where, if we measured the marginal bone position from baseline to T2, we saw marginal bone resorption but at T2 the marginal bone position was always coronal to the implant platform and never exposing the implant. In other implants scenario 2 occurred where the marginal bone position at T0 was coronal to the implant platform but at T2 the marginal bone position was apical to the implant platform, leaving the implant collar exposed to oral environment (meaning that the bone was not covering part of the implant collar).

Measure A (MBL1) was referred to as the total amount of marginal bone loss (MBL1) that occurred from T0 to T2 in all implants of the study, independent of whether the bone stayed above or below the implant platform. This measurement was done from the most coronal part of the bone crest to the

implant platform or where the first implant to bone contact occurred (if there was bone loss apical to the platform). In this type of measurement, total amount of bone loss was included, even in cases where there was no bone loss apical to the implant platform (in those that lost bone but stayed coronal to the implant platform).

Measure A (also called in this work MBL1) was calculated as follows: Each calibrated investigator calculated the mesial and distal marginal bone loss for each implant/healing abutment complex. From this, an average of the mesial and distal values was caluclated.

Since there were 3 independent readings the mean average of the three reading in each abutment was calculated.

Finally, the mean average of the mesial and distal result was caluclated.

Marginal bone loss is a 3D phenomenon that on a radiograph only has two dimensions. By calculating the mean average of the mesial and distal result, an average result of the resorption pattern that occurs in each implant is obtained.

We felt that the implants that still retained coronal bone, despite experiencing bone loss, were not as clinically exposed to the oral environment as the ones that lost bone apical to the implant platform. With this in mind, **Measure B** or MBL2 was created to attribute the value “0 bone loss” to the implants that were able to maintain bone coronal to the implant platform margin.

In this study, the results were always contrasted with the two items MBL1 or measure A and MBL2 or measure B.

Measurement on the computer was done according to the following methodology:

Calculation of MBL. A line, referred to as the “implant platform line” was drawn connecting the vertices of the mesial and distal platform, and was represented with the number 3 in fig. 126. Point 1 and 2 represent the most coronal point of bone in contact with the implant abutment. Accordingly, the measurement was made by drawing a line from the implant platform line to point 1 and 2 for mesial and distal measurements.

This is the position of marginal bone at T0.

At T2 the same platform line was drawn, and the same point chosen for the coronal bone (nº4 and 5 of fig. 127) and the amount of available bone was measured.

The marginal bone resorption involved a calculation of the difference between the marginal bone crest at T0 and at T2. Note that in fig. 128 there is a radiological feature that corresponds to remodelled bone that does not leave implant platform exposed.

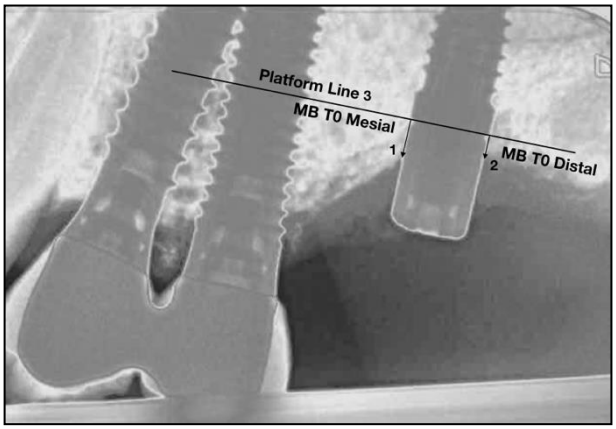


FIGURE 145 - Calculation of MBL A. A line was drawn connecting the vertices of the mesial and distal platform. The line is called the implant platform line and is represented with ar 3 in the image. Point 1 and 2 represent the most coronal point of the bone in contact with the implant abutment. The measurement is made by drawing a line from the implant platform line to point 1 and 2 for mesial and distal measures. This is the position of marginal bone at T0.

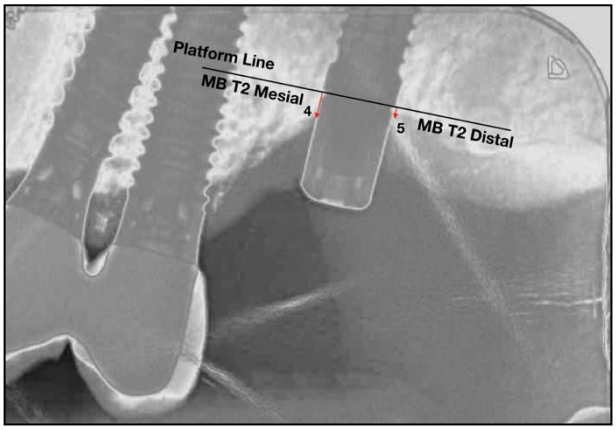


FIGURE 146 - At T2 the same platform line and the same point for the coronal bone (nº 4 and 5) was drawn and the amount of available bone measured.

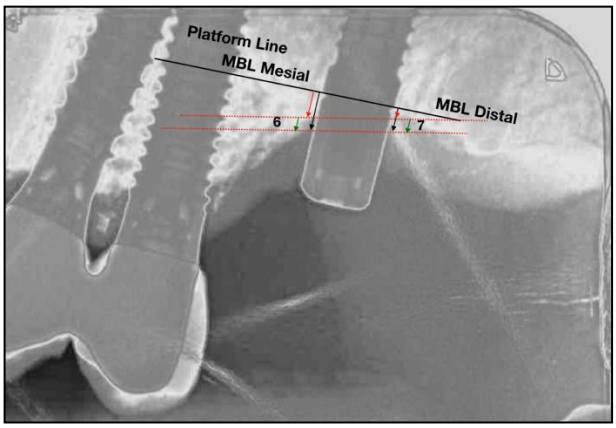


FIGURE 147 - Marginal bone resorption calculation. This is the difference between the marginal bone crest at T0 and at T2. Note that this case illustrates a radiological feature that corresponds to a remodeled bone that does not leave implant platform exposed.

Measure B (also referred to in this work as MBL2) is when, if the implant was not exposed, the MBL is taken as 0, while only counting values in those implants that had marginal bone loss apically to the implant platform.

In the calculation of implants where there was marginal bone position at T2 apical to implant platform as a radiological feature, a line was drawn

connecting the vertices of the mesial and distal platform. This line was referred to as the “implant platform line” and was represented with a 3 on image 129. Point 1 and 2 represented the most coronal point of bone in contact with the implant abutment. In the case of fig. 130 the mesial segment of the implant lost more bone than the distal. Point 4 of fig. 130 is now apical to the implant platform and has a negative value.

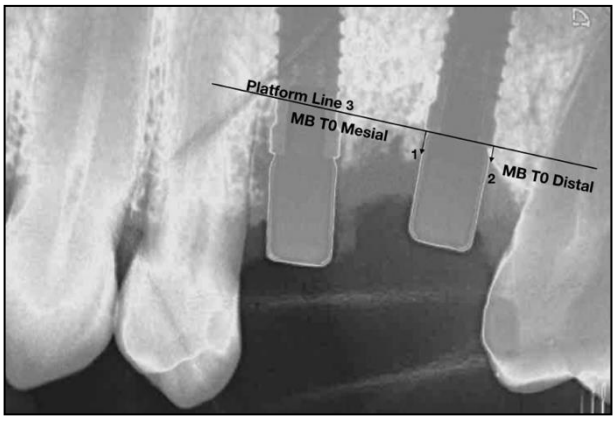


FIGURE 148 - Measure B: a line was drawn connecting the vertices of the mesial and distal platform. The line is referred to as the “implant platform line” and is represented with number 3 on the image. Point 1 and 2 represent the most coronal point of bone in contact with the implant abutment.

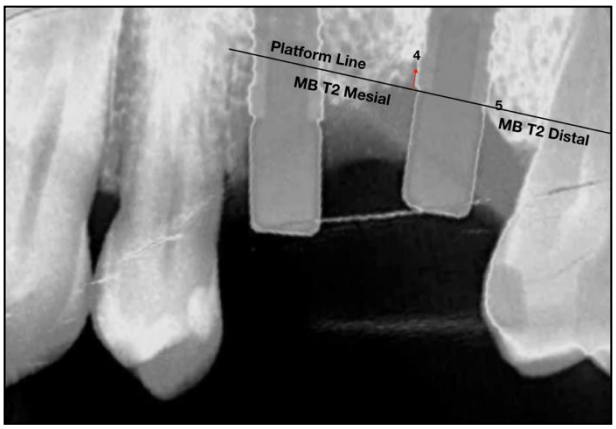


FIGURE 149 - View of MBL. In this case the mesial segment of the implant, lost more bone than the distal. Point 4 is now apical to the implant platform and has a negative value.

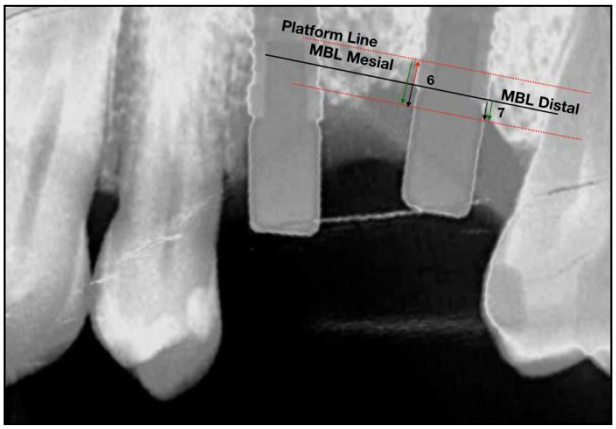


FIGURE 150 - Marginal bone resorption calculation. Is the difference between marginal bone crest at T0 and at T2. Note that this case illustrates the radiological feature that corresponds to remodeled bone that, when placed, exposes the implant platform and therefore may have a different impact on the surrounding tissues.

We devised this approach because we wanted to correlate the inflammation pattern to the presence of different biomaterials. Since the implants were not exposed to the titanium and not in contact with soft tissues there was no marginal bone resorption exposing the implant. On the contrary, if there had been marginal bone resorption and the implant had been exposed, then it would have been important to study the inflammation pattern that occurred on those implants and if they had an impact on marginal bone resorption.

Specific aim 1: Considering a general overview

H0: There is no difference between Marginal Bone Loss (MBL) in titanium healing abutments compared to zirconia and acrylic, in implants placed under the standard protocol, at T2 (8 weeks).

H1: There is a difference between Marginal Bone Loss (MBL) in titanium healing abutments compared to zirconia and acrylic, in implants placed under the standard protocol, at T2 (8 weeks).

Consider the group of CAD-CAM Zirconia healing abutment at T2 (8 weeks):

Specific aim 2: considering the total amounts of interleukins (IL-1β +IL6) present at peril-implant sulcus:

H0: There is no correlation between Marginal Bone Loss (MBL) and the total number of interleukins (IL-1β+IL6) in implants placed under the standard protocol at T2 (8 weeks) in the zirconia healing abutment group.

H1: There is correlation between Marginal Bone Loss (MBL) and the total number of interleukins (IL-1β+IL6) in implants placed under the standard protocol at T2 (8 weeks) in the zirconia healing abutment group.

Specific aim 3: considering the total amounts of interleukin IL6 present at peri-implant sulcus

H0: There is no correlation between Marginal Bone Loss (MBL) and the total amount of interleukin IL6 in implants placed under the standard protocol at T2 (8 weeks) in the zirconia healing abutment group.

H1: There is correlation between Marginal Bone Loss (MBL) and the total amount of interleukin IL6 in implants placed under the standard protocol at T2 (8 weeks) in the zirconia healing abutment group.

Specific aim 4: considering the total amounts of interleukin IL-1β present at peri-implant sulcus

H0: There is no correlation between Marginal Bone Loss (MBL) and the total amount of interleukin IL-1β in implants placed under the standard protocol at T2 (8 weeks) in the zirconia healing abutment group.

H1: There is a correlation between Marginal Bone Loss (MBL) and the total amount pf interleukin IL-1β in implants placed under the standard protocol at T2 (8 weeks) in the zirconia healing abutment group.

Considering the group of CAD-CAM Titanium healing abutment at T2 (8 weeks):

Specific aim 5: considering the total amounts of interleukins (IL-1β +IL6) present at the peri-implant sulcus

H0: There is no correlation between Marginal Bone Loss (MBL) and the total amount of interleukin (IL-1β+IL6) in implants placed under the standard protocol at T2 (8 weeks) in the titanium healing abutment group.

H1: There is correlation between marginal bone loss (MBL) and the total amount of interleukin (IL-1β+IL6) in implants placed under the standard protocol at T2 (8 weeks) in the titanium healing abutment group.

Specific aim 6: considering the total amounts of interleukin IL6 present at peri-implant sulcus

H0: There is no correlation between marginal bone loss (MBL) and the total amount of interleukin IL6 in implants placed under the standard protocol, in the titanium healing abutment group.

H1: There is correlation between marginal bone loss (MBL) and the total amount of interleukin IL6 in implants placed under the standard protocol in the titanium healing abutment group.

Specific aim 7: considering the total amounts of interleukin IL-1β present at peri-implant sulcus,

H0: There is no correlation between marginal bone loss (MBL) and the total amount of interleukin IL-1β in implants placed under the standard protocol in the titanium healing abutment group.

H1: There is a correlation between marginal bone loss (MBL) and the total amount of interleukin IL-1β in implants placed under the standard protocol in the titanium healing abutment group.

Considering the group of CAD-CAM Acrylic healing abutment at T2 (8 weeks):

Specific aim 8: considering the total amounts of interleukins (IL-1β+IL6) present at peri-implant sulcus,

H0: There is no correlation between marginal bone loss (MBL) and the total amount of interleukins (IL-1β+IL6) in implants placed under the standard protocol in the acrylic healing abutment group.

H1: There is correlation between marginal bone loss (MBL) and the total amount of interleukins (IL-1β+IL6) in implants placed under the standard protocol in the acrylic healing abutment group

Specific aim 9: considering the total amounts of interleukin IL6 present at peri-implant sulcus,

H0: There is no correlation between marginal bone loss (MBL) and the total amount of interleukin IL6 in implants placed under the standard protocol in the acrylic healing abutment group.

H1: There is a correlation between marginal bone loss (MBL) and the total amount of interleukin IL6 in implants placed under the standard protocol in the acrylic healing abutment group

Specific aim 10: considering the total amounts of interleukin IL-1β **present at peri-implant sulcus**

H0: There is no correlation between marginal bone loss (MBL) and the total amount of interleukin IL-1β in implants placed under the standard protocol in the acrylic healing abutment group

H1: There is a correlation between marginal bone loss (MBL) and the total amount of interleukin IL-1β in implants placed under the standard protocol in the acrylic healing abutment group

Correlation Between Inflammation Vs Abutment Material (Z, T, A) vs Marginal Bone Loss (Measure B). Correlation of the amount of marginal bone resorption with the concentrations of interleukins present in T1 dependent on the abutment material used.

Specific aim 1: Considering de general overview

H0: There is no difference between marginal bone loss (MBL2) in titanium healing abutments compared to zirconia and acrylic, in implants placed under the standard protocol.

H1 There is a difference between marginal bone loss (MBL2) in titanium healing abutments compared to zirconia and acrylic, in implants placed under the standard protocol.

Considering the group of CAD-CAM Zirconia healing abutment at T2 (8 weeks):

Specific aim 2: considering the total amounts of interleukins (IL-1β+IL6) present at peri-implant sulcus

H0: There is no correlation between marginal bone loss (MBL2) and the total amount of interleukins (IL-1β+IL6) in implants placed under the standard protocol in the zirconia healing abutment group.

H1: There is a correlation between marginal bone loss (MBL2) and the total amount of interleukin (IL-1β+IL6) in implants placed under the standard protocol in the zirconia healing abutment group

Specific aim 3: considering the total amounts of interleukin IL6 present at peri-implant sulcus

H0: There is no correlation between marginal bone loss (MBL2) and the total amount of interleukin IL6 in implants placed under the standard protocol in the zirconia healing abutment group.

H1: There is a correlation between marginal bone loss (MBL2) and the total amount of interleukin IL6 in implants placed under the standard protocol in the zirconia healing abutment group.

Specific aim 4: considering the total amounts of interleukin IL-1β present at the peri-implant sulcus,

H0: There is no correlation between marginal bone loss (MBL2) and the total amount of interleukin IL-1β in implants placed under the standard protocol in the zirconia healing abutment group.

H1: There is a correlation between marginal bone loss (MBL2) and the total amount of interleukin IL-1β in implants placed under the standard protocol in the zirconia healing abutment group

Considering the group of CAD-CAM Titanium healing abutment at T2 (8 weeks):

Specific aim 5: considering the total amounts of interleukins (IL-1β +IL6) present at the peri-implant sulcus,

H0: There is no correlation between marginal bone loss (MBL2) and the total amount of interleukins (IL-1β+IL6) in implants placed under the standard protocol in the titanium healing abutment group.

H1: There is a correlation between marginal bone loss (MBL2) and the total amount of interleukins (IL-1β+IL6) in implants placed under the standard protocol in the titanium healing abutment group

Specific aim 6: considering the total amounts of interleukin IL6 present at peri-implant sulcus

H0: There is no correlation between marginal bone loss (MBL2) and the total amount of interleukin IL6 in implants placed under the standard protocol in the titanium healing abutment group.

H1: There is a correlation between marginal bone loss (MBL2) and the total amount interleukin IL6 in implants placed under the standard protocol in the the titanium healing abutment group

Specific aim 7: considering the total amounts of interleukin IL-1β present at peri-implant sulcus

H0: There is no correlation between marginal bone loss (MBL2) and the total amount of interleukin IL-1β in implants placed under the standard protocol in the titanium healing abutment group

H1: There is a correlation between marginal bone loss (MBL2) and the total amount of interleukin IL-1β in implants placed under the standard protocol in the titanium healing abutment group

Consider the group of CAD-CAM Acrylic healing abutment at T2 (8 weeks):

Specific aim 8: considering the total amounts of interleukins (IL-1β+IL6) present at the peri-implant sulcus,

H0: There is no correlation between marginal bone loss (MBL2) and the total amount of interleukins (IL-1β+IL6) in implants placed under the standard protocol in the acrylic healing abutment group.

H1: There is a correlation between marginal bone loss (MBL2) and the total amount of interleukins (IL-1β+IL6) in implants placed under the standard protocol in the acrylic healing abutment group.

Specific aim 9 considering the total amounts of interleukin IL6 present at the peri-implant sulcus,

H0: There is no correlation between marginal bone loss (MBL2) and the total amount of interleukin IL6 in implants placed under the standard protocol in the acrylic healing abutment group

H1: There is a correlation between marginal bone loss (MBL2) and the total amount of interleukin IL6 in implants placed under the standard protocol in the acrylic healing abutment group.

Specific aim 10: considering the total amounts of interleukin IL-1β present at the peri-implant sulcus,

H0: There is no correlation between marginal bone loss (MBL2) and the total amount of interleukin IL-1β in implants placed under the standard protocol in the acrylic healing abutment group.

H1: There is a correlation between marginal bone loss (MBL2) and the total amount of interleukin IL-1β in implants placed under the standard protocol in the acrylic healing abutment group.

Section 5.4.2 Results

Each implant/abutment complex was measured at T0 and then at T2 to ascertain the difference in bone resorption. Table 80 and table 81 represent the raw number found for each investigator and the respective mean.

Table 82 represents the marginal bone remodeling calculated from the known values T0 and T2.

Table 80 - Overall results of mesial and distal marginal bone level at T0 (baseline). Three measurements by Three independent investigators. Mean average.

			Bone Level at T0 in mm											
#	T	M	MB T0 1			MB T0 2			MB T0 3			MB T0 Total		
			M	D	Ave	M	D	Ave	M	D	Ave	M	D	(M+d) /2
1	47	A	3	9	6	3	10	6,5	2	9	5,5	2,67	9,33	6,00
2	45	A	15	16	15,5	17	16	16,5	17	16	16,5	16,33	16,00	16,17
3	25	A	17	29	23	26	27	26,5	24	31	27,5	22,33	29,00	25,67
4	16	Z	17	30	23,5	9	27	18	10	31	20,5	12,00	29,33	20,67
5	36	T	1	0	0,5	3	0	1,5	2	0	1	2,00	0,00	1,00
6	25	T	12	14	13	13	14	13,5	13	14	13,5	12,67	14,00	13,33
7	37	T	0	6	3	0	8	4	0	7	3,5	0,00	7,00	3,50
8	37	A	0	0	0	0	0	0	0	0	0	0,00	0,00	0,00
9	46	A	3	8	5,5	0	8	4	0	8	4	1,00	8,00	4,50
10	17	T	11	0	5,5	8	0	4	12	0	6	10,33	0,00	5,17
11	24	A	10	11	10,5	10	11	10	12	10	11	10,67	10,67	10,50
12	22	A	30	36	33	28	32	30	27	38	32,5	28,33	35,33	31,83
13	36	Z	25	12	18,5	22	11	16,5	23	11	17	23,33	11,33	17,33
14	26	T	21	0	10,5	21	2	11,5	21	3	12	21,00	1,67	11,33
15	46	Z	11	6	8,5	10	6	8	11	6	8,5	10,67	6,00	8,33
16	11	Z	21	15	18	29	14	21,5	24	17	20,5	24,67	15,33	20,00
17	25	T	25	27	26	26	28	27	24	30	27	25,00	28,33	26,67
18	36	Z	15	0	7,5	17	0	8,5	18	0	9	16,67	0,00	8,33
19	15	Z	15	0	7,5	15	0	7,5	16	0	8	15,33	0,00	7,67
20	37	T	10	5	7,5	10	3	6,5	10	5	7,5	10,00	4,33	7,17
21	46	Z	6	4	5	6	4	5	6	5	5,5	6,00	4,33	5,17
22	16	A	4	0	2	4	0	2	3	0	1,5	3,67	0,00	1,83
23	26	T	17	7	12	20	9	14,5	19	8	13,5	18,67	8,00	13,33
24	14	Z	6	17	11,5	6	18	12	6	18	12	6,00	17,67	11,83
25	14	Z	13	10	11,5	11	10	10,5	11	10	10,5	11,67	10,00	10,83
26	24	T	31	5	18	29	4	16,5	31	4	17,5	30,33	4,33	17,33
27	25	A	12	12	12	12	14	13	12	11	11,5	12,00	12,33	12,17
28	15	T	19	13	16	23	14	18,5	20	14	17	20,67	13,67	17,17
29	36	Z	0	3	1,5	0	3	1,5	0	2	1	0,00	2,67	1,33
30	36	A	10	6	8	9	5	7	9	5	7	9,33	5,33	7,33

31	24	T	4	18	11	4	19	11,5	5	19	12	4,33	18,67	11,50
32	46	T	0	0	0	0	0	0	0	0	0	0,00	0,00	0,00
33	46	Z	0	5	2,5	0	5	2,5	0	4	2	0,00	4,67	2,33
34	16	Z	3	0	1,5	4	0	2	4	0	2	3,67	0,00	1,83
35	24	T	14	29	21,5	14	24	19	15	24	19,5	14,33	25,67	20,00
36	45	A	0	2	1	0	4	2	0	3	1,5	0,00	3,00	1,50
37	26	T	23	12	17,5	19	11	15	21	10	15,5	21,00	11,00	16,00
38	24	Z	14	6	10	13	8	10,5	11	7	9	12,67	7,00	9,83
39	16	A	19	17	18	27	25	26	26	23	24,5	24,00	21,67	22,83
40	25	Z	20	15	17,5	18	13	15,5	18	13	15,5	18,67	13,67	16,17
41	15	Z	8	2	5	2	7	4,5	2	6	4	4,00	5,00	4,50
42	23	Z	0	6	3	4	5	4,5	2	4	3	2,00	5,00	3,50
43	14	Z	8	26	17	11	26	18,5	8	24	16	9,00	25,33	17,17
44	14	A	8	21	14,5	8	18	13	8	13	10,5	8,00	17,33	12,67
45	36	A	10	0	5	8	0	4	8	0	4	8,67	0,00	4,33
46	25	Z	6	6	6	5	5	5	4	4	4	5,00	5,00	5,00
47	24	A	31	14	22,5	34	16	25	34	15	24,5	33,00	15,00	24,00
48	35	T	Early Implant Loss											
49	45	T	18	0	9	18	0	9	18	0	9	18,00	0,00	9,00
50	15	A	11	8	9,5	11	10	10,5	11	10	10,5	11,00	9,33	10,17
51	44	T	8	0	4	8	2	5	8	0	4	8,00	0,67	4,33
52	24	A	21	21	21	22	28	25	21	24	22,5	21,33	24,33	22,83
53	25	T	9	8	8,5	9	11	10	7	8	7,5	8,33	9,00	8,67
54	26	Z	14	9	11,5	15	7	11	14	7	10,5	14,33	7,67	11,00
55	25	Z	26	17	21,5	28	19	23,5	29	17	23	27,67	17,67	22,67
56	46	Z	Early Implant Loss											
57	15	A	9	8	8,5	8	7	7,5	10	7	8,5	9,00	7,33	8,17
58	35	A	5	4	4,5	6	4	5	7	4	5,5	6,00	4,00	5,00
59	12	Z	15	9	12	4	12	8	4	12	8	7,67	11,00	9,33
60	34	A	11	0	5,5	11	0	5,5	12	0	6	11,33	0,00	5,67

Table 81 - Overall results of mesial and distal marginal bone level at T2 (baseline). Three measurements by Three independent investigators. Mean average

Bone Level at T2 in mm														
#	T	M	MBL After 1			MBL After 2			MBL After 3			MBL after total		
			M	D	Ave	M	D	Ave	M	D	Ave	M	D	(M+d)/2

1	47	A	-24	-20	-22	-17	-15	-16	-17	-14	-15,5	-19,33	-16,33	-17,83
2	45	A	15	15	15	17	15	16	17	16	16,5	16,33	15,33	15,83
3	25	A	10	12	11	10	15	12,5	11	13	12	10,33	13,33	11,83
4	16	Z	3	23	13	5	21	13	7	29	18	5,00	24,33	14,67
5	36	T	2	-4	-1	2	-4	-1	3	0	1,5	2,33	-2,67	-0,17
6	25	T	10	7	8,5	9	6	7,5	9	6	7,5	9,33	6,33	7,83
7	37	T	0	-5	-2,5	4	-4	0	0	-4	-2	1,33	-4,33	-1,50
8	37	A	-6	-6	-6	-7	-7	-7	-7	-7	-7	-6,67	-6,67	-6,67
9	46	A	-17	-11	-14	-21	-18	-19,5	-16	-13	-14,5	-18,00	-14,00	-16,00
10	17	T	10	0	5	6	0	3	9	0	4,5	8,33	0,00	4,17
11	24	A	10	2	6	10	0	11	9	0	4,5	9,67	0,67	7,17
12	22	A	26	27	26,5	26	28	27	27	27	27	26,33	27,33	26,83
13	36	Z	21	9	15	22	8	15	20	8	14	21,00	8,33	14,67
14	26	T	13	0	6,5	14	0	7	15	0	7,5	14,00	0,00	7,00
15	46	Z	-9	-7	-8	-13	-8	-10,5	-10	-8	-9	-10,67	-7,67	-9,17
16	11	Z	20	15	17,5	20	11	15,5	20	14	17	20,00	13,33	16,67
17	25	T	22	24	23	22	25	23,5	20	22	21	21,33	23,67	22,50
18	36	Z	-15	-8	-11,5	-16	-8	-12	-15	-7	-11	-15,33	-7,67	-11,50
19	15	Z	15	-3	6	17	-4	6,5	15	-3	6	15,67	-3,33	6,17
20	37	T	10	3	6,5	10	2	6	11	3	7	10,33	2,67	6,50
21	46	Z	-6	-2	-4	-4	-4	-4	-4	-1	-2,5	-4,67	-2,33	-3,50
22	16	A	-19	-17	-18	-19	-18	-18,5	-19	-18	-18,5	-19,00	-17,67	-18,33
23	26	T	15	3	9	14	4	9	15	4	9,5	14,67	3,67	9,17
24	14	Z	-8	-9	-8,5	-8	-7	-7,5	-11	-7	-9	-9,00	-7,67	-8,33
25	14	Z	8	7	7,5	7	7	7	7	5	6	7,33	6,33	6,83
26	24	T	-12	-35	-23,5	-12	-40	-26	-12	-42	-27	-12,00	-39,00	-25,50
27	25	A	12	12	12	12	14	13	12	11	11,5	12,00	12,33	12,17
28	15	T	19	13	16	23	14	18,5	19	13	16	20,33	13,33	16,83
29	36	Z	0	-5	-2,5	0	-6	-3	0	-6	-3	0,00	-5,67	-2,83
30	36	A	-3	-11	-7	-3	-11	-7	-6	-14	-10	-4,00	-12,00	-8,00
31	24	T	0	10	5	-3	19	8	-2	19	8,5	-1,67	16,00	7,17
32	46	T	-15	-12	-13,5	-17	-13	-15	-15	-14	-14,5	-15,67	-13,00	-14,33
33	46	Z	-9	0	-4,5	-7	2	-2,5	-8	1	-3,5	-8,00	1,00	-3,50
34	16	Z	2	0	1	3	0	1,5	2	0	1	2,33	0,00	1,17
35	24	T	0	-13	-6,5	0	-11	-5,5	0	-12	-6	0,00	-12,00	-6,00
36	45	A	-10	-12	-11	-6	-10	-8	-12	-11	-11,5	-9,33	-11,00	-10,17
37	26	T	20	8	14	18	3	10,5	19	3	11	19,00	4,67	11,83

38	24	Z	14	2	8	16	2	9	13	1	7	14,33	1,67	8,00
39	16	A	10	15	12,5	17	18	17,5	16	20	18	14,33	17,67	16,00
40	25	Z	6	3	4,5	6	4	5	0	5	2,5	4,00	4,00	4,00
41	15	Z	-12	-19	-15,5	-8	-17	-12,5	-8	-9	-8,5	-9,33	-15,00	-12,17
42	23	Z	0	6	3	4	5	4,5	2	4	3	2,00	5,00	3,50
43	14	Z	8	21	14,5	10	24	17	8	21	14,5	8,67	22,00	15,33
44	14	A	5	9	7	8	10	9	8	13	10,5	7,00	10,67	8,83
45	36	A	-9	-11	-10	-10	-9	-9,5	-13	-13	-13	-10,67	-11,00	-10,83
46	25	Z	0	-3	-1,5	0	-4	-2	-1	-2	-1,5	-0,33	-3,00	-1,67
47	24	A	25	4	14,5	26	8	17	25	9	17	25,33	7,00	16,17
48	35	T	Early Implant Loss											
49	45	T	-4	-12	-8	-4	-11	-7,5	-5	-11	-8	-4,33	-11,33	-7,83
50	15	A	11	-15	-2	4	-18	-7	6	-19	-6,5	7,00	-17,33	-5,17
51	44	T	-10	-11	-10,5	-8	-10	-9	-8	-13	-10,5	-8,67	-11,33	-10,00
52	24	A	21	21	21	19	21	20	21	21	21	20,33	21,00	20,67
53	25	T	-19	-23	-21	-18	-19	-18,5	-17	-26	-21,5	-18,00	-22,67	-20,33
54	26	Z	0	7	3,5	0	7	3,5	0	4	2	0,00	6,00	3,00
55	25	Z	10	-9	0,5	10	-5	2,5	6	-7	-0,5	8,67	-7,00	0,83
56	46	Z	Early Implant Loss											
57	15	A	-3	-3	-3	-3	-2	-2,5	-3	-4	-3,5	-3,00	-3,00	-3,00
58	35	A	-5	-6	-5,5	-5	-5	-5	-7	-6	-6,5	-5,67	-5,67	-5,67
59	12	Z	-12	-9	-10,5	-11	-7	-9	-11	-9	-10	-11,33	-8,33	-9,83
60	34	A	-8	-11	-9,5	-7	-9	-8	-7	-10	-8,5	-7,33	-10,00	-8,67

Table 82 - Overall results of mesial and distal marginal bone loss (MBL) Three measurements by Three independent investigators. Mean average.

Implant #	Tooth #	Material	Marginal Bone Loss (T2-T0) Diff M	D	Ave	Implant Exposure
1	47	A	22,00	25,67	23,83	23,83

2	45	A	0,00	0,67	0,33	0
3	25	A	12,00	15,67	13,83	0
4	16	Z	7,00	5,00	6,00	0
5	36	T	-0,33	2,67	1,17	1,17
6	25	T	3,33	7,67	5,50	0
7	37	T	-1,33	11,33	5,00	5,00
8	37	A	6,67	6,67	6,67	6,67
9	46	A	19,00	22,00	20,50	20,50
10	17	T	2,00	0,00	1,00	0
11	24	A	1,00	10,00	3,33	0
12	22	A	2,00	8,00	5,00	0
13	36	Z	2,33	3,00	2,67	0
14	26	T	7,00	1,67	4,33	0
15	46	Z	21,33	13,67	17,50	17,50
16	11	Z	4,67	2,00	3,33	0
17	25	T	3,67	4,67	4,17	0
18	36	Z	32,00	7,67	19,83	19,83
19	15	Z	-0,33	3,33	1,50	0
20	37	T	-0,33	1,67	0,67	0
21	46	Z	10,67	6,67	8,67	8,67
22	16	A	22,67	17,67	20,17	20,17
23	26	T	4,00	4,33	4,17	0
24	14	Z	15,00	25,33	20,17	20,17
25	14	Z	4,33	3,67	4,00	0
26	24	T	42,33	43,33	42,83	42,83
27	25	A	0,00	0,00	0,00	0
28	15	T	0,33	0,33	0,33	0
29	36	Z	0,00	8,33	4,17	4,17
30	36	A	13,33	17,33	15,33	15,33
31	24	T	6,00	2,67	4,33	0
32	46	T	15,67	13,00	14,33	14,33
33	46	Z	8,00	3,67	5,83	5,83
34	16	Z	1,33	0,00	0,67	0
35	24	T	14,33	37,67	26,00	26,00
36	45	A	9,33	14,00	11,67	11,67
37	26	T	2,00	6,33	4,17	0
38	24	Z	-1,67	5,33	1,83	0
39	16	A	9,67	4,00	6,83	0

40	25	Z	14,67	9,67	12,17	0
41	15	Z	13,33	20,00	16,67	16,67
42	23	Z	0,00	0,00	0,00	0
43	14	Z	0,33	3,33	1,83	0
44	14	A	1,00	6,67	3,83	0
45	36	A	19,33	11,00	15,17	23,83
46	25	Z	5,33	8,00	6,67	6,67
47	24	A	7,67	8,00	7,83	0
48	35	T	EarlyImplantLoss			
49	45	T	22,33	11,33	16,83	16,83
50	15	A	4,00	26,67	15,33	15,33
51	44	T	16,67	12,00	14,33	14,33
52	24	A	1,00	3,33	2,17	0
53	25	T	26,33	31,67	29,00	29,00
54	26	Z	14,33	1,67	8,00	0
55	25	Z	19,00	24,67	21,83	0
56	46	Z	EarlyImplantLoss			
57	15	A	12,00	10,33	11,17	11,17
58	35	A	11,67	9,67	10,67	10,67
59	12	Z	19,00	19,33	19,17	19,17
60	34	A	18,67	10,00	14,33	14,33

Fig. 132 to 134 provides an overview of the parallel methodology used for radiographic acquisition and interpretation. Acrylic, titanium and zirconia are shown at T0 and T2. As we can see, within the limitations of the technique, there is some pararellism between them.

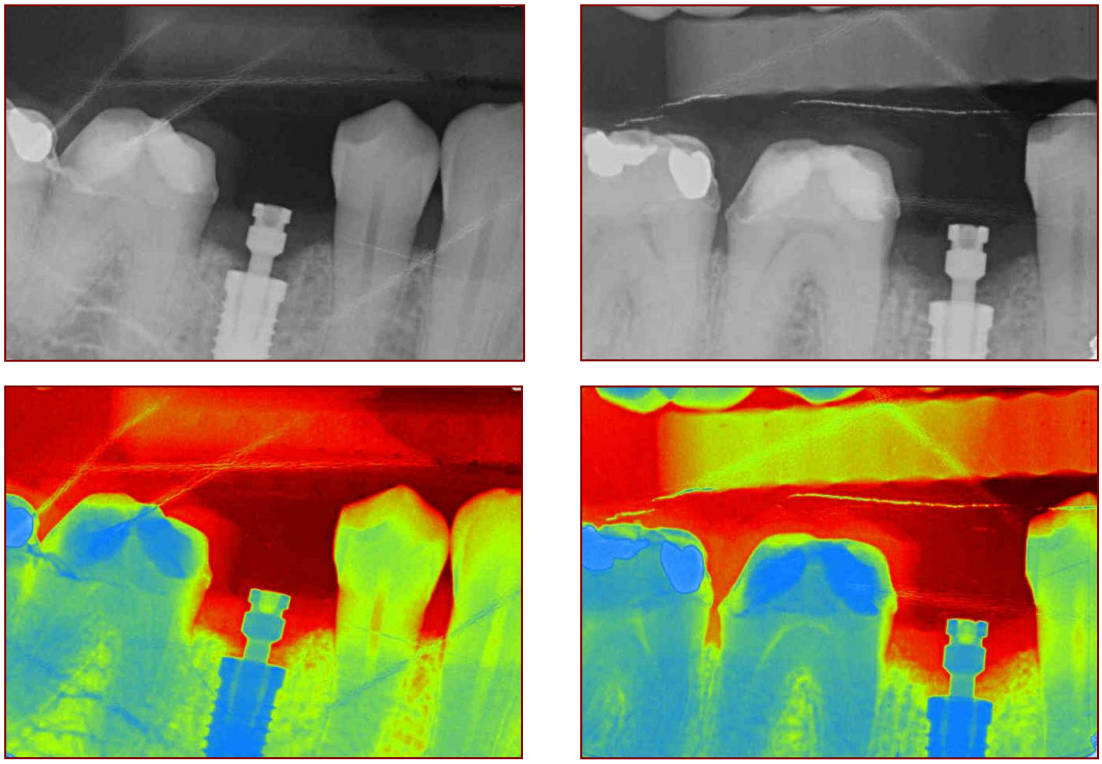


FIGURE 151 - Acrylic healing abutment display at T0 baseline and at T2 (8 weeks). Marginal Bone loss reading protocol.

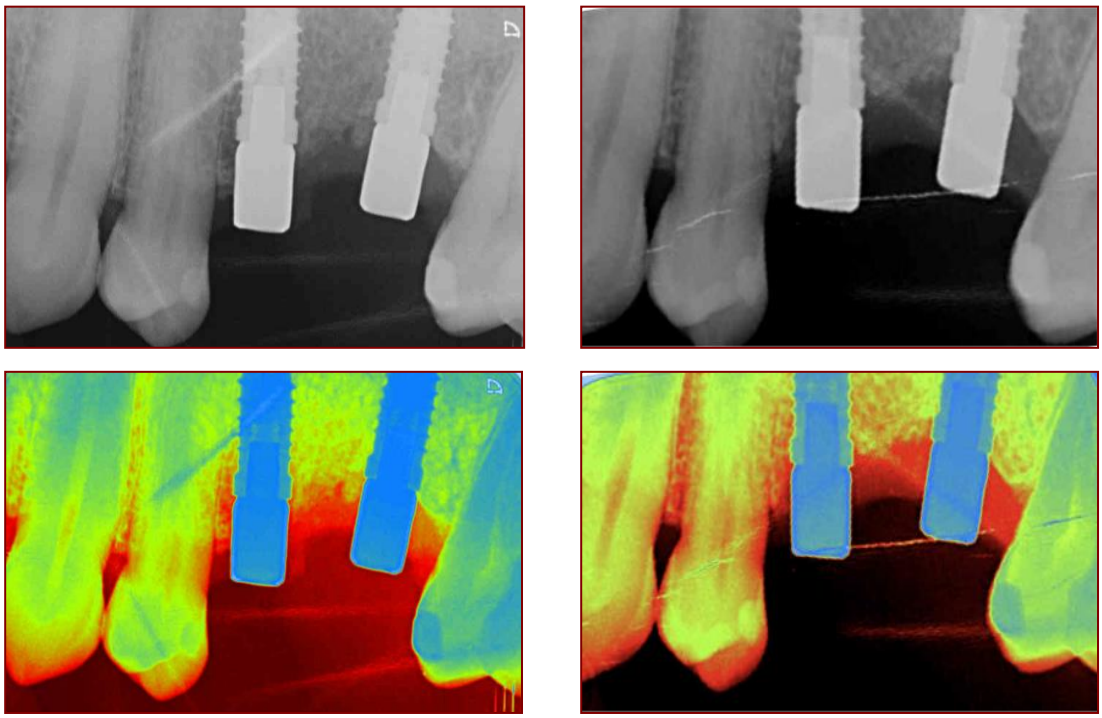


FIGURE 152 - Zirconia healing abutment display at T0 baseline and at T2 (8 weeks). Marginal Bone loss reading protocol

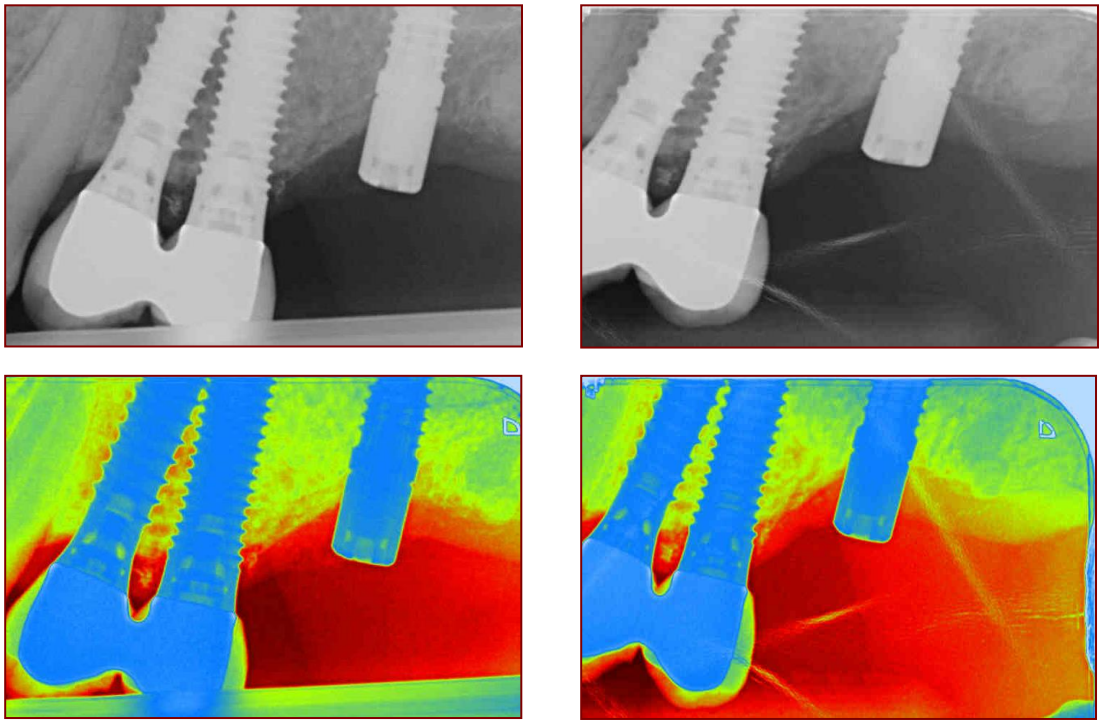


FIGURE 153 - Zirconia healing abutment display at T0 baseline and at T2 (8 weeks). Marginal Bone loss reading protocol

Overall Sample size for Marginal bone loss reading, the chart on fig. 135 represents the total n number per healing abutment that was used for MBL calculation and interpretation.

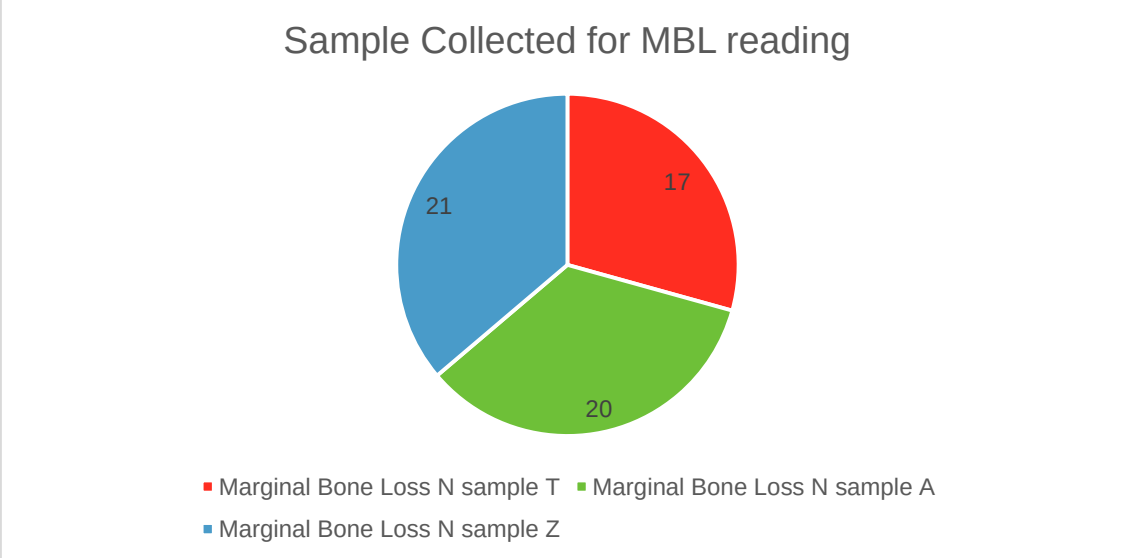


FIGURE 154 - Overall Sample size for Marginal bone loss reading. The chart represents the total n number per healing abutment.

Mean marginal bone loss independent of the material reported a mean value of 9,8 mm in measure A and a mean value of 7,61 mm in measure B (that is, that if the implant collar was not exposed that the MBL is considered as 0), as shown in table 136.

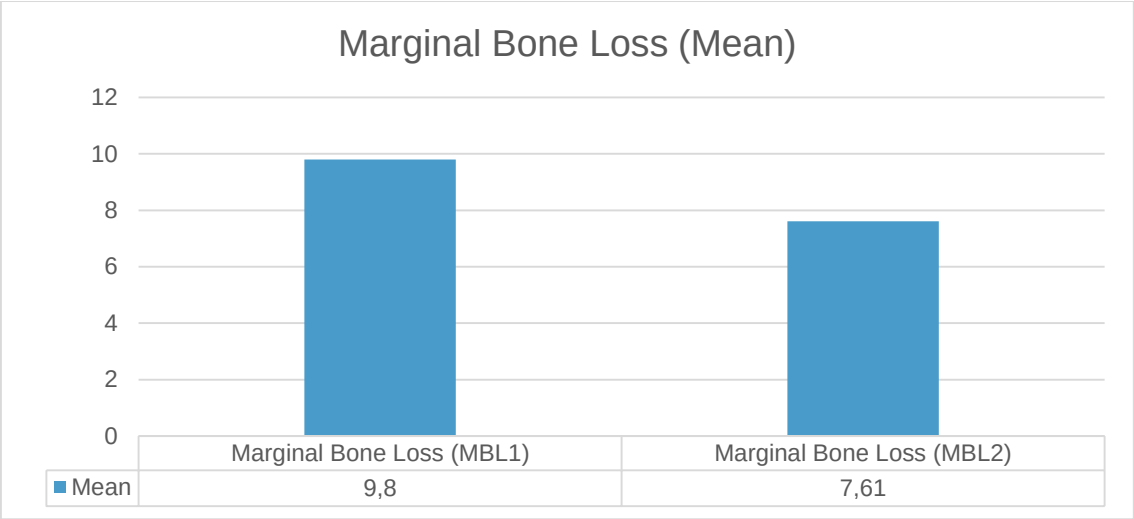


FIGURE 155 - Marginal Bone Loss Total represents the overall bone resorption from T0 to T2, Marginal Bone Loss (exposure) represents overall marginal bone loss only to implants whose collar exposed above the bone. For this last statistic the non-exposed implants received 0 mm in MBL but were entered into the final MBL statistic.

When the marginal bone loss was divided by the healing abutment biomaterial there was a tendency for zirconia to display less marginal bone loss that the others. The results are shown in table 137.

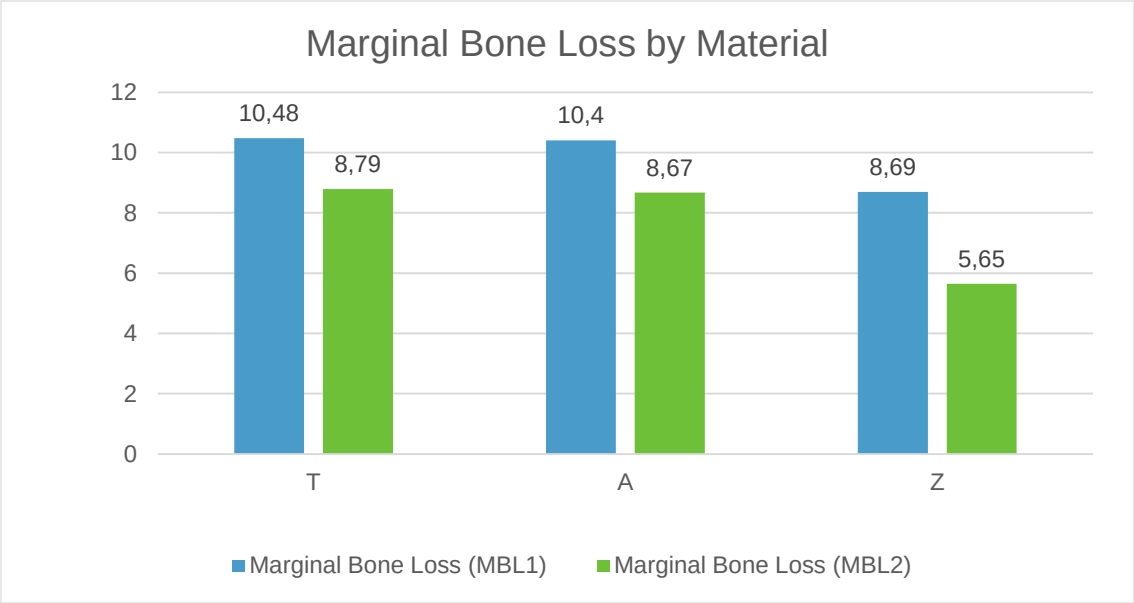


FIGURE 156 - Marginal Bone Loss expressed by different Biomaterials (A, Z and T). Note that there is a tendency for there to be less marginal bone loss when a zirconia healing abutment is used as compared to titanium and acrylic.

Although the tendency was for the zirconia healing abutment to induce less marginal bone resorption there were no statistically significant differences found between either in measure A (MBL1) or measure B (MBL2) as shown in table 83.

Table 83 - Hypothesis and statistical conclusions of the relationship between Marginal Bone Loss (MBL) and Biomaterial (Z, A, T)

MBL	Test	Null Hypothesis	P-Value
MBL 1* Vs (Z, A, T)	kruskall-Wallis	Retain	0,677
MBL 2" Vs (Z, A, T)	kruskall-Wallis	Retain	0,626

*MBL 1 – is the overall marginal bone loss

"MBL 2 – in the overall marginal bone loss of the implants exposed, the non-exposed received 0 but count for the overall mean.

The second indicator that we looked for was if there was any correlation between marginal bone loss and inflammatory levels. The results showed a tendency for there to be less MBL in the Z healing abutment, and there also seems to be a correlation where less IL-1β is expressed, as shown by the mean averages and SD found (table 84).

In this work, no correlation was found between MBL (measured at T2) and the initial concentration on IL measured at baseline. None of the three biomaterials correlated more or less to marginal bone resorption as shown in table 85 and figure 138.

Table 84 - Summary of mean Marginal Bone Loss and Interleukin Concentrations

Material	Marginal Bone Loss (MBL1)	Marginal Bone Loss (MBL2)	IL6pg/ml T0	IL-1β pg/ml T0	IL6+IL1βpg/ml T0
T	10,48 ± 12,05	8,79 ± 13,13	4,65±4,57	6,35 ± 5,37	11 ± 8,59
A	10,40 ± 6,92	8,67 ± 9,04	7,63±6,58	5,31 ± 3,16	12,95 ±7,78
Z	8,69 ± 7,43	5,65± 7,91	6,12±4,64	4,11 ± 2,7	10,28 ± 6,6

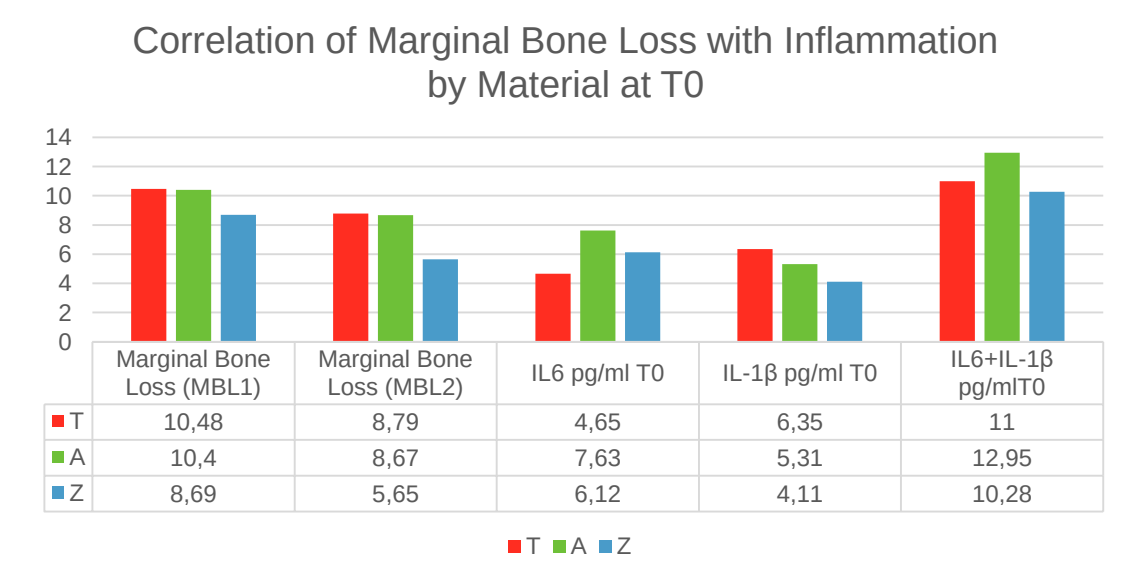


FIGURE 157 - Overall Marginal bone loss (MBL1 and MBL2) and the correlation between IL6, IL-1β and total IL concentrations at T0.

There was no correlation found at T2 between inflammatory levels and MBL, as shown in table 85.

Table 85 - Summary of mean on Marginal Bone Loss and Interleukins Concentrations

Material	Marginal Bone Loss (MBL1)	Marginal Bone Loss (MBL2)	IL6 pg/ml T2	IL-1β pg/ml T2	IL6 IL-1β pg/ml T2
T	10,48 ± 12,05	8,79 ± 13,13	4,06 ±7,99	64,75 ±55,24	68,81 ± 59,81
A	10,40 ± 6,92	8,67 ± 9,04	8,56 ±14,82	31,44 ±33,40	40 ± 39,66
Z	8,69 ± 7,43	5,65± 7,91	4,76 ±13,83	29,94 ±54,07	34,70 ± 55,99

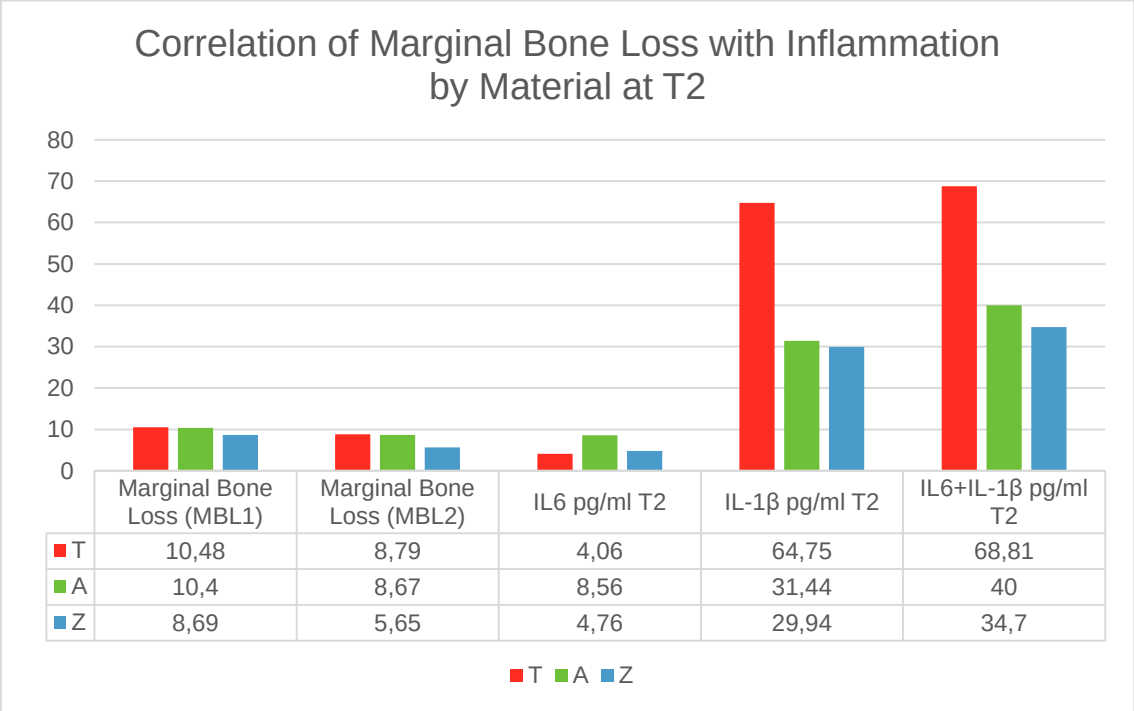


FIGURE 158 - Overall Marginal bone loss (MBL1 and MBL2) and the correlation between IL6, IL-1β and total IL concentrations at T2.

Statistical test and results for for Cad-Cam Acrylic Healing abutment:

Table 86 - Correlations between MBL, Interleukin Levels in the Acrylic Material

	Test	IL6	IL-1β	Total
MBL1	Pearson Correlation	,261	,435	,201
MBL2	Pearson Correlation	,261	,814	,295

Statistical test and results for For Cad-Cam Titanium Healing abutment:

Table 87 - Correlations between MBL, Interleukin Levels in the Titanium Material

	Test	IL6	IL-1β	Total
MBL1	Pearson Correlation	0,710	0,779	0,982
MBL2	Pearson Correlation	0,769	0,865	0,960

Statistical test and results for For Cad-Cam Zirconia Healing abutment:

Table 88 - Correlations between MBL, Interleukin Levels in the Zirconia Material

	Test	IL6	IL-1β	Total
MBL1	Pearson Correlation	0,163	0,675	0,432
MBL2	Pearson Correlation	0,060	0,701	0,146

SECTION 5.5 SECONDARY OUTCOME MEASURES: ZIRCONIA, ACRYLIC AND TITANIUM INFLAMMATION LEVELS OF IL6 AND IL-1β AND CORRELATION TO MARGINAL BONE LOSS AND HEIGHT OF EXISTING TISSUE AT THE TIME OF SURGERY (BIOLOGICAL WIDTH HEIGHT)- HYPOTHESIS AND RESULTS

Section 5.5.1. Hypothesis

Correlation between Height of tissue (2 Vs 3 mm), marginal bone loss and inflammation.

Specific aim 1: To measure general overall Height Vs MBL. At T2 (8 weeks)

H0: There is no difference between marginal bone loss (MBL), in implants placed in 2 mm of pre-existing biological width (connective and epithelium) and implants placed in 3 mm of pre-existing biological width, under the standard protocol.

H1: There is a difference between marginal bone loss (MBL), in implants placed in 2 mm of pre-existing biological width (connective and epithelium) and implants placed in 3 mm of pre-existing biological width, under the standard protocol.

Considering general overview Height Vs Inflammation

Specific aim 2: At Baseline T0.

For Total IL levels:

H0: There is no correlation between Gingival Height (2mm Vs 3 mm) and inflammatory levels (IL6+IL-1β).

H1: There is a correlation between Gingival Height (2mm Vs 3 mm) and inflammatory levels (IL6+IL-1β).

For IL6 levels:

H0: There is no correlation between Gingival Height (2mm Vs 3 mm) and inflammatory levels (IL6).

H1: There is a correlation between Gingival Height (2mm Vs 3 mm) and inflammatory levels (IL6).

For IL-1β Levels:

H0: There is no correlation between Gingival Height (2mm Vs 3 mm) and inflammatory levels (1β).

H1: There is a correlation between Gingival Height (2mm Vs 3 mm) and inflammatory levels (IL-1β).

Specific aim 3: At T2 (8 Weeks)

For Total IL levels:

H0: There is no correlation between Gingival Height (2mm Vs 3 mm) and inflammatory levels (IL6+IL-1β).

H1: There is a correlation between Gingival Height (2mm Vs 3 mm) and inflammatory levels (IL6+IL-1β).

For IL6 levels:

H0: There is no correlation between Gingival Height (2mm Vs 3 mm) and inflammatory levels (IL6).

H1: There is a correlation between Gingival Height (2mm Vs 3 mm) and inflammatory levels (IL6).

For IL-1β Levels:

H0: There is no correlation between Gingival Height (2mm Vs 3 mm) and inflammatory levels (IL-1β).

H1: There is a correlation between Gingival Height (2mm Vs 3 mm) and inflammatory levels (IL-1β).

Section 5.5.2. Results

Each implant/abutment complex was measured at T0 for gingival height of tissue (biological width). Table 89 and table 90 represent the raw number found for height of tissue and the respective mean and SD. On the right side of the table MBL numbers are displayed for comparison.

Table 89 - Overall results of Abutment material, Biologic width and their correlation with inflammation and marginal bone loss. Mean average

Implant #	Tooth #	Material	Height of Tissue	MBL MBL1	MBL MBL2
1	47	A	3	23,83	23,83
2	45	A	3	0,33	0
3	25	A	3	13,83	0
4	16	Z	2	6,00	0
5	36	T	2	1,17	1,17
6	25	T	3	5,50	0
7	37	T	3	5,00	5,00
8	37	A	3	6,67	6,67
9	46	A	2	20,50	20,50
10	17	T	4	1,00	0
11	24	A	3	3,33	0
12	22	A	3	5,00	0
13	36	Z	1	2,67	0
14	26	T	1	4,33	0
15	46	Z	3	17,50	17,50
16	11	Z	3	3,33	0
17	25	T	2	4,17	0
18	36	Z	2	19,83	19,83
19	15	Z	3	1,50	0
20	37	T	3	0,67	0
21	46	Z	2	8,67	8,67
22	16	A	3	20,17	20,17
23	26	T	3	4,17	0
24	14	Z	2	20,17	20,17

25	14	Z	3	4,00	0
26	24	T	2	42,83	42,83
27	25	A	3	0,00	0
28	15	T	3	0,33	0
29	36	Z	2	4,17	4,17
30	36	A	3	15,33	15,33
31	24	T	3	4,33	0
32	46	T	2	14,33	14,33
33	46	Z	3	5,83	5,83
34	16	Z	3	0,67	0
35	24	T	3	26,00	26,00
36	45	A	3	11,67	11,67
37	26	T	2	4,17	0
38	24	Z	2	1,83	0
39	16	A	3	6,83	0
40	25	Z	3	12,17	0
41	15	Z	3	16,67	16,67
42	23	Z	4	0,00	0
43	14	Z	3	1,83	0
44	14	A	3	3,83	0
45	36	A	3	15,17	23,83
46	25	Z	3	6,67	6,67
47	24	A	3	7,83	0
48	35	T	Early Implant Loss		
49	45	T	3	16,83	16,83
50	15	A	3	15,33	15,33
51	44	T	2	14,33	14,33
52	24	A	2	2,17	0
53	25	T	2	29,00	29,00
54	26	Z	3	8,00	0
55	25	Z	3	21,83	0
56	46	Z	Early Implant Loss		
57	15	A	3	11,17	11,17
58	35	A	2	10,67	10,67
59	12	Z	2	19,17	19,17
60	34	A	3	14,33	14,33

Sample size for statistical data extraction is shown in fig. 139.

Sample size and distribution, for statistical reading to analyze correlation between biological width and inflammatory levels.

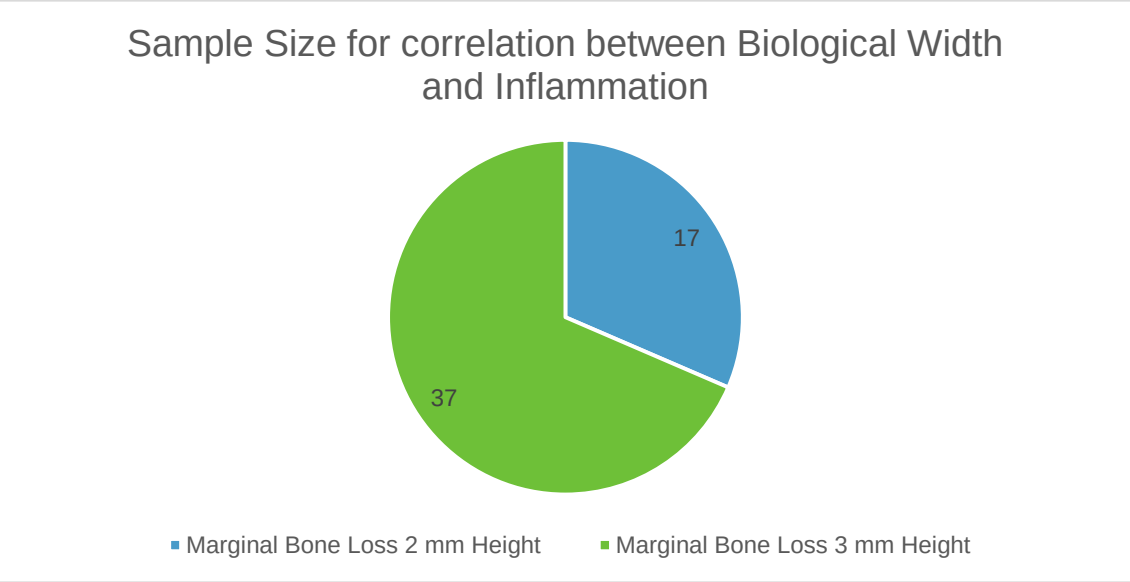


FIGURE 159 - Sample size and distribution, for statistical reading to analyze correlation between biological width and inflammatory levels.

Correlation Between Height of Tissue and Marginal Bone Resorption

The mean marginal bone loss and SD found in each tissue height are displayed in table 90. One could draw the conclusion that gingival height does not significantly influence marginal bone loss, either in measure MBL1 or MBL2.

Table 90 - Summary of mean on Marginal Bone Loss at T2 and Height of Tissue

Heigh Tissue	Marginal Bone Loss (MBL1)	Marginal Bone Loss (MBL2)
2mm	13,13 ± 11,26	12,04 ± 12,28
3mm	9,12 ± 7,25	6,40 ± 8,59

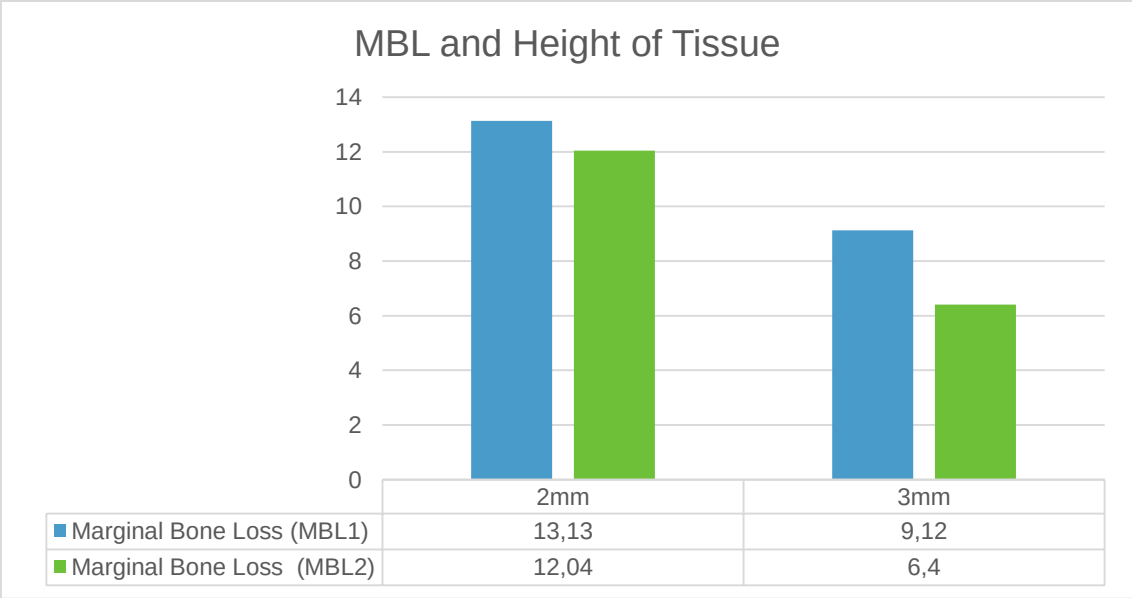


FIGURE 160 - Correlation values between pre-existing height of tissue at T0 and marginal bone resorption at T2

If we correlate the height of tissue by interleukin and time frame the following results, shown in table 91 and fig. 141 were observed.

Table 91 - Mean average of concentration of Interleukins and height of tissue

Height of tissue in mm	IL6 T0	IL6 T2	IL-1β T0	IL-1β T2	IL6+IL1β T0	IL6+ IL1β T0
2	2,87	9,67	4,25	46,67	7,13	56,33
	± 4,03	± 16,76	± 4,68	± 50,97	± 7,01	± 55,70
3	7,41	4,36	5,50	38,76	12,91	43,12
	± 5,40	± 10,75	± 3,53	± 52,01	± 7,25	± 54,74

From fig. 139 we can see that the sample was uneven, since there were 37 implants that had 3 mm of preexisting gingiva against only 17 that had 2 mm. (the difference was reflected in the statistical methodology comparing the different items)

In table 90 we see a summary of the correlation between MBL and height of tissue and the tendency to have less MBL when the height was greater than 2mm. This showsing that implants that were placed with 3 mm of preexisting gingiva had a a tendency to express less MBL.

In fact, when the value 0 mm of MBL are attributed to those implants that didn't lose bone apical to the implant platform (measure b or MBL2) the difference

goes from 12,04 mm to 6,4 mm.

To correlate these two variables, the non-parametric Mann-Whitney test for clinical significance was used and in fact, although the tendency is there, in this study we cannot say conclusively that the greater MBL found for the 2mm of preexisting gingiva was statistically relevant when compared to the 3 mm.

Correlation Between Height of Tissue and Inflammatory Patterns

When we compared IL levels with height of tissue at T0 we can see in fig. 145 that all concentration values were higher with 3 mm tissue height (IL6- 7,41 pg/ml, IL-1β -5,5 pg/ml and total 12,91 pg/ml) than for 2mm (IL6-2,87 pg/ml, IL-1β -4,25 pg/ml and total 7,13 pg/ml). There's was a statistically significant difference (Mann Whitney) between all the IL6, IL-1β and total at T0 when we compared implants placed in 2 mm of preexisting gingiva and implants placed in 3 mm of preexisting gingiva, expressing higher values in the 2mm heights. (table 93)

At T2 the tendency was exactly the opposite, showing that IL values, were higher in the 2 mm of preexisting gingiva (IL6 -9,67 pg/ml, IL-1β -46,67 pg/ml and total 56,33 pg/ml) than the 3-mm group (IL6 -4,36 pg/ml, IL-1β -38,76 pg/ml and total -43,12 pg/ml)

Although there was a tendency, we could not find any correlation between the rise of IL levels, height of preexisting tissue and marginal bone resorption (table 94).

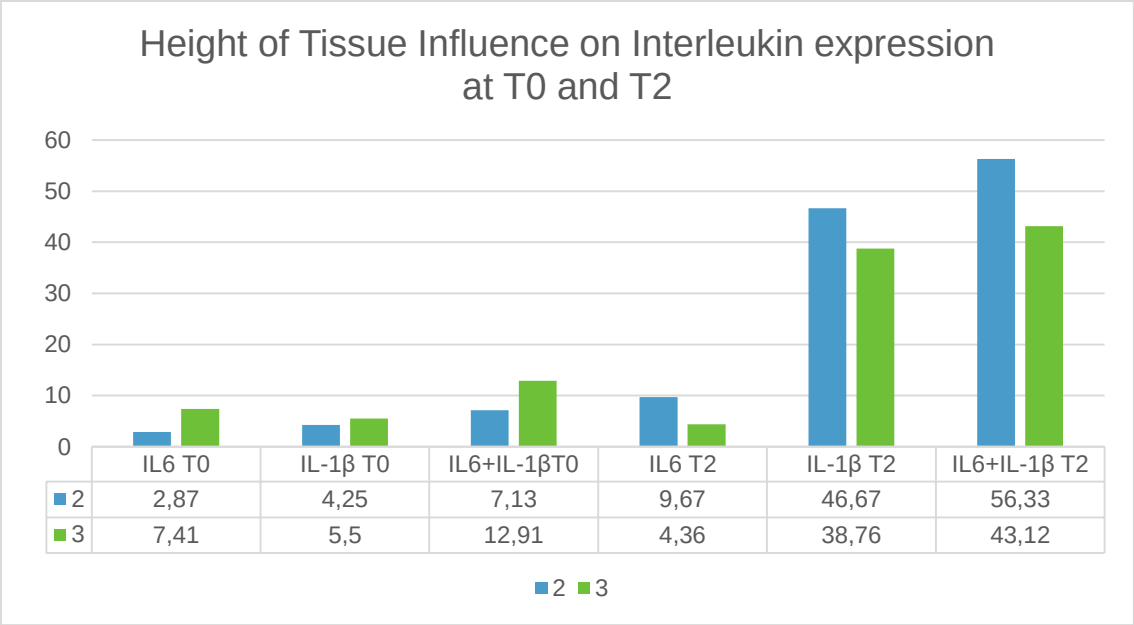


FIGURE 161 - Comparison of Interleukin variation by height of tissue (2 or 3 mm) and at each time frame T0 vs T2.

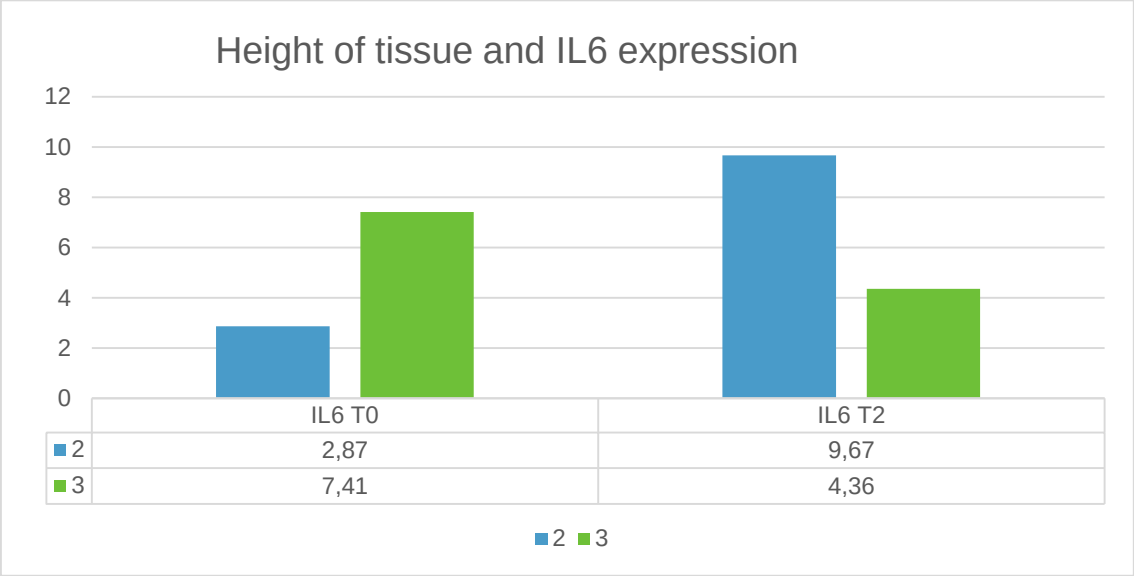


FIGURE 162 - Overall correlation between height of tissue and IL6 variation in different time frames.

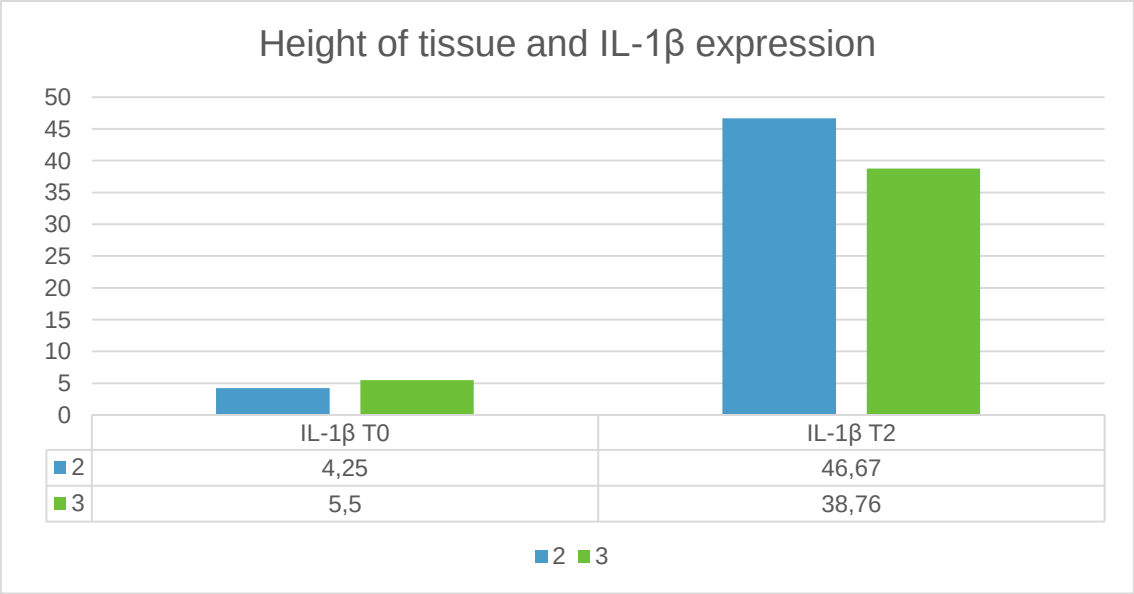


FIGURE 163 - Overall correlation between height of tissue and IL-1β variation at different time frames.

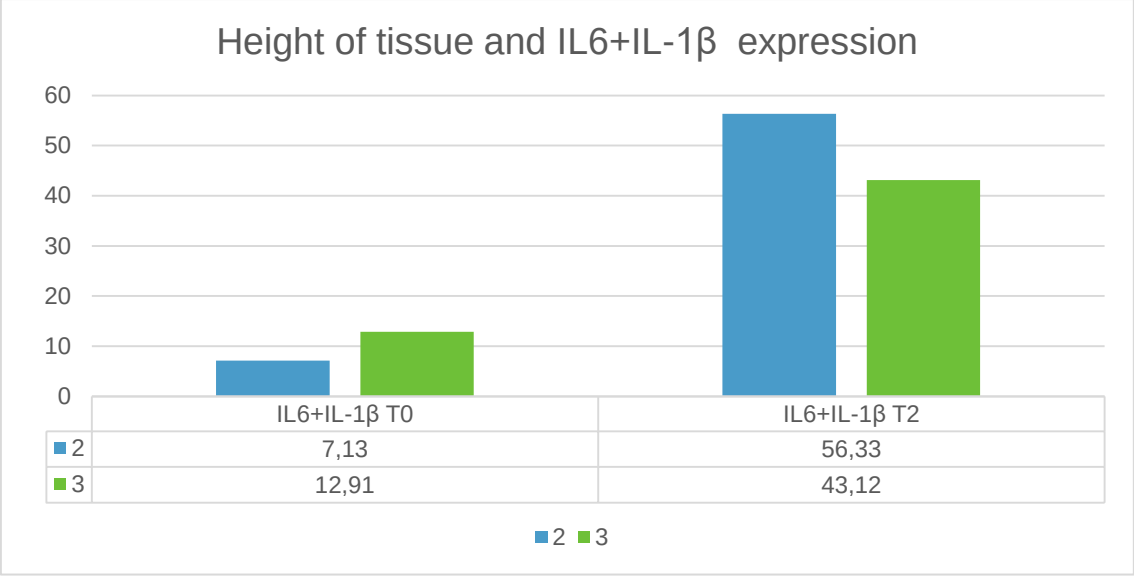


FIGURE 164 - Overall correlation between height of tissue and IL6+IL-1β variation at different time frames

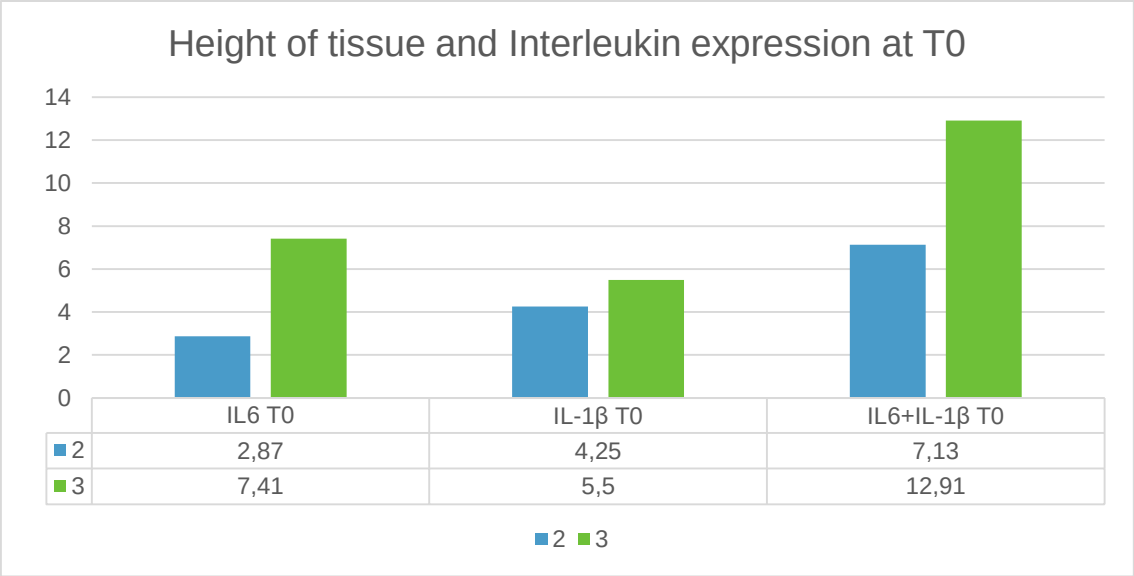


FIGURE 165 - Overall correlation between height of tissue and IL6, IL-1β and total at T0 Baseline

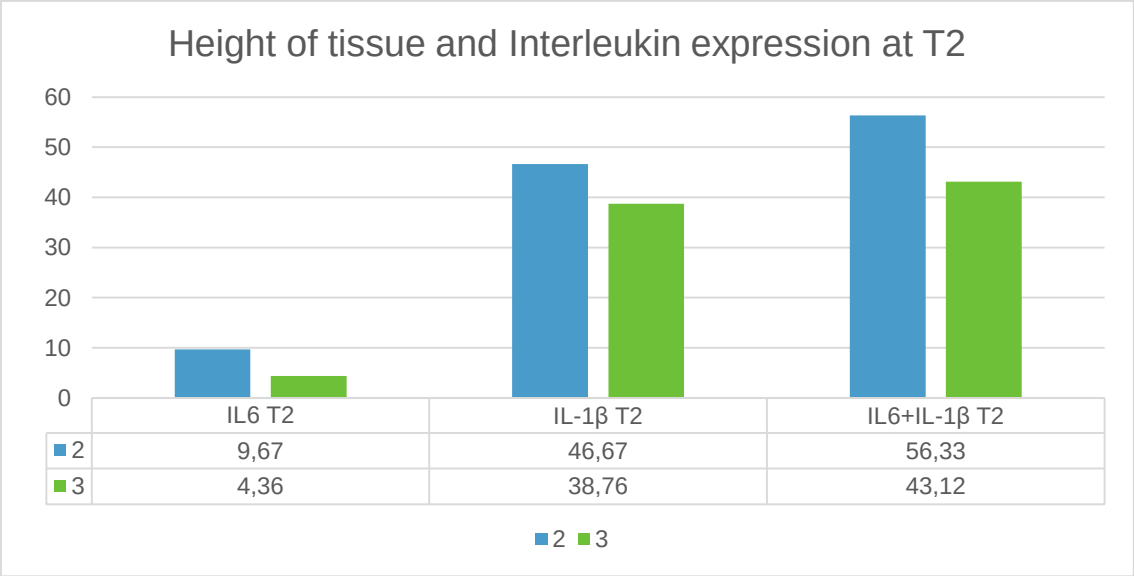


FIGURE 166 - Overall correlation between height of tissue, IL6, IL-1β and total variation at T2

Table 92 - Correlations between MBL (MBL1 and MBL2) and Height Tissue (2 or 3mm)

MBL	Test	P-Value	Correlation
MBL1 with 2/3 mm height	Mann-Whitney	,264	No
MBL2 with 2/3 mm height	Mann-Whitney	,068	No

Table 93 - Correlations between Interleukin Levels and Height Tissue (2 or 3mm) at T0

Interleukin (IL)	Test	*P*-Value	Correlation
IL-1β with 2/3 mm height at T0	Mann-Whitney	0,025	Yes
IL6 with 2/3 mm height at T0	T-Test	,004	Yes
IL6+IL-1β with 2/3 mm height at T0	Mann-Whitney	0,009	Yes

Table 94 - Correlations between Interleukin Levels and Height Tissue (2 or 3mm) at T2

Interleukin (IL)	Test	*P*-Value	Correlation
IL-1β with 2/3 mm height at T2	Mann-Whitney	0,578	No
IL6 with 2/3 mm height at T2	Mann-Whitney	0,463	No
IL6+IL-1β with 2/3 mm height at T2	Mann-Whitney	0,370	No

SECTION 5.6. SECONDARY OUTCOME MEASURES: ZIRCONIA, ACRYLIC AND TITANIUM INFLAMMATION LEVELS OF IL6 AND IL-1β AND CORRELATION TO MARGINAL BONE LOSS AND AGE - HYPOTHESIS AND RESULTS

Section 5.6.1. Hypothesis

Correlation between Age, marginal bone loss (MBL1, MBL2) and inflammation levels (IL-1β, IL6 and total IL)

With age as the central variable and independent of the abutment material placed two groups were considered: those younger than 65 (<65) and those older or equal to 65 (≥65) years old):

Relate Age and Marginal Bone loss (MBL1 and MBL2), independent of the material.

Specific aim 1: To relate age to Marginal Bone Loss (MBL) Overall at T2 (8 weeks).

H0: There is no difference between age in the two given groups and marginal bone loss in implants placed under the standard protocol.

H1: There is a difference between age in the two given groups and marginal bone loss in implants placed under the standard protocol.

Relate Age and Inflammatory Levels

Specific aim 2: To relate to Inflammatory Levels Overall (IL-1β +IL6) at T2 (8 weeks)

H0: There is no difference between age in the two given groups and overall inflammatory levels (IL-1β+IL6) in implants placed under the standard protocol.

H1: There is a difference between age in the two given groups and overall inflammatory levels (IL-1β+IL6) in implants placed under the standard protocol.

Specific aim :3 To relate age to Inflammatory Levels Interleukin IL-1β At T2 (8 weeks).

H0: There is no difference between age (in the two given groups) and inflammatory levels (IL-1β) in implants placed under the standard protocol.

H1: There is a difference between age (in the two given groups) and inflammatory levels (IL-1β) in implants placed under the standard protocol.

Specific aim 4: To relate age to Inflammatory Levels Interleukin IL6 at T2 (8 weeks).

H0: There is no difference between age in the two given groups and inflammatory levels (IL6) in implants placed under the standard protocol.

H1: There is a difference between age in the two given groups and inflammatory levels (IL6) in implants placed under the standard protocol.

Specific aim 5: To relate age to Inflammatory Levels Overall (IL-1β +IL6) at T0 (Baseline).

H0: There is no difference between age in the two given groups and overall inflammatory levels (IL-1β+IL6) in implants placed under the standard protocol.

H1: There is a difference between age in the two given groups and overall inflammatory levels (IL-1β+IL6) in implants placed under the standard protocol.

Specific aim 6: To relate age to Inflammatory Levels Interleukin IL-1β at T0 (Baseline).

H0: There is no difference between age in the two given groups and inflammatory levels (IL-1β) in implants placed under the standard protocol.

H1: There is a difference between age in the two given groups and inflammatory levels (IL-1β) in implants placed under the standard protocol.

Specific aim 7: To relate age to Inflammatory Levels Interleukin IL6 at T0 (Baseline).

H0: There is no difference between age in the two given groups and inflammatory levels (IL6) in implants placed under the standard protocol.

H1: There is a difference between age (in the two given groups) and inflammatory levels (IL6) in implants placed under the standard protocol.

Section 5.6.2. Results

In this chapter, we wanted to correlate inflammatory levels with age and marginal bone loss. Table 95 shows the raw data collected divided by age and material used.

Table 95 - Mean average of concentration of Interleukins and Age

Implant #	Tooth #	Material	Age
1	47	A	32
2	45	A	57
3	25	A	47
4	16	Z	72
5	36	T	63
6	25	T	68
7	37	T	66
8	37	A	68
9	46	A	63
10	17	T	42
11	24	A	61
12	22	A	73
13	36	Z	66
14	26	T	87
15	46	Z	57
16	11	Z	53
17	25	T	36
18	36	Z	62
19	15	Z	62

20	37	T	37
21	46	Z	36
22	16	A	62
23	26	T	68
24	14	Z	57
25	14	Z	79
26	24	T	57
27	25	A	73
28	15	T	65
29	36	Z	62
30	36	A	48
31	24	T	73
32	46	T	54
33	46	Z	79
34	16	Z	79
35	24	T	40
36	45	A	79
37	26	T	54
38	24	Z	59
39	16	A	58
40	25	Z	61
41	15	Z	68
42	23	Z	74
43	14	Z	71
44	14	A	80
45	36	A	72
46	25	Z	43
47	24	A	68
48	35		
49	45	T	57
50	15	A	40
51	44	T	72
52	24	A	65
53	25	T	65
54	26	Z	54
55	25	Z	54
56	46		
57	15	A	59

58	35	A	63
59	12	Z	53
60	34	A	57

Fig. 147 represents the healing abutments that were integrated for data extraction and statistical value treatment to compare inflammation/marginal bone loss and age. We see that the groups are unevenly distributed since we have more samples in the over 65 age group than the under 65 (35 Vs 23).

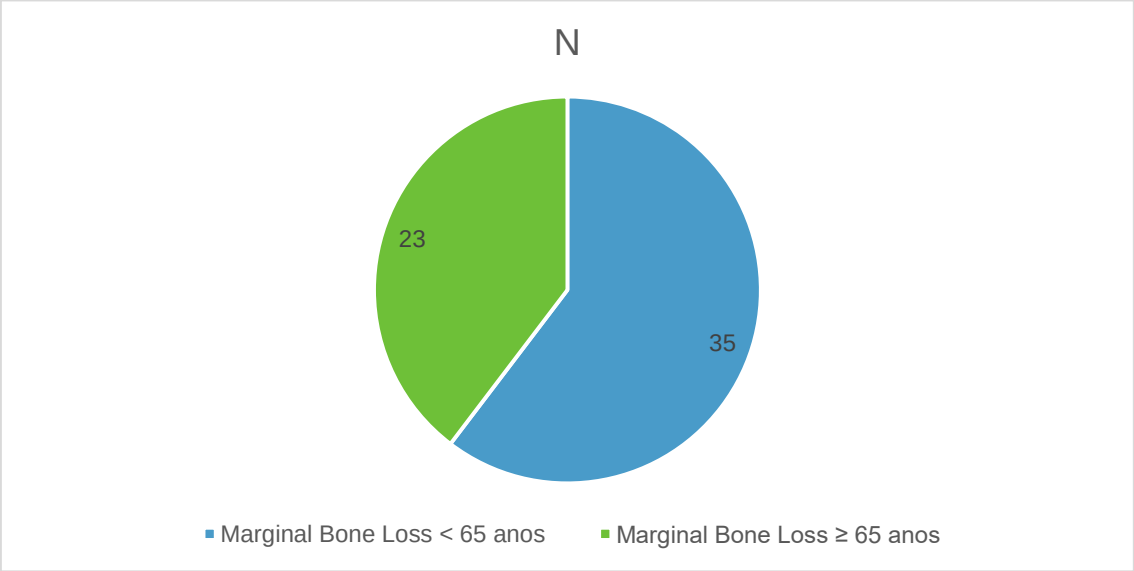


FIGURE 167 - Sample size and distribution for statistical reading to analyze correlation between Age and inflammatory levels

Correlation Between Gender and Marginal Bone Resorption

In Fig. 148, when age was correlated to MBL a tendency was exhibited for people under 65 years to show less marginal bone loss than people older than 65 (11,93 mm against 9,39 mm – table 96).

There was in fact a statistically significant difference in the marginal bone loss pattern which was higher in people under 65 years old, although when we ascribed “0 values” (measure b) to those implants where there was no lost bone apical to the implant platform there was no observed difference. (table 97)

Table 96 represents the mean average and SD found in each group.

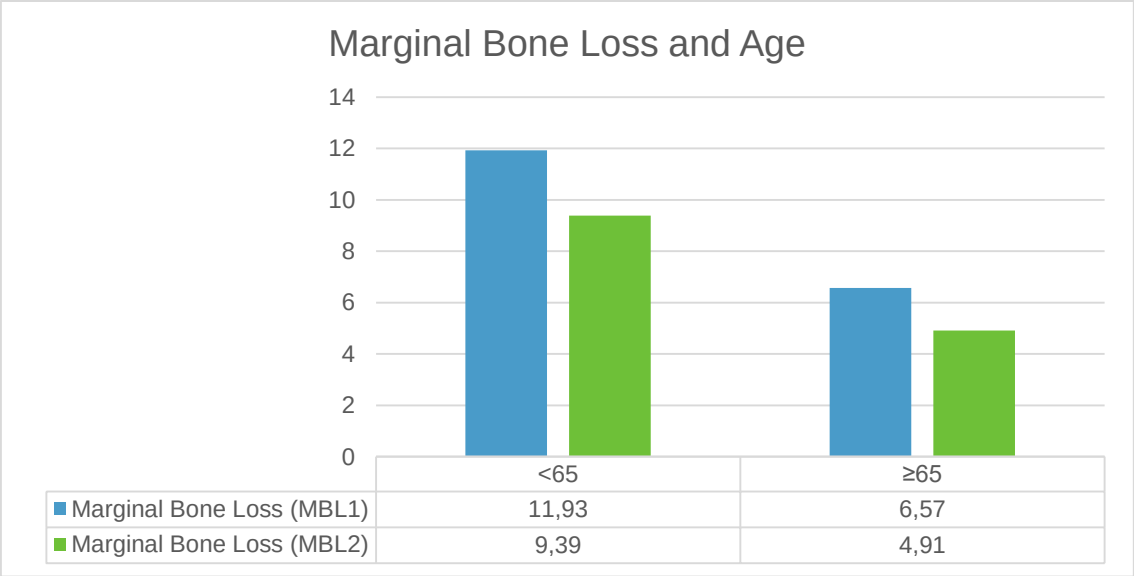


FIGURE 168 - Comparison MBL1 and MBL2 with Age

Table 96 - Mean average of concentration of Interleukins, marginal bone loss and Age

Age	MBL1	MBL2	IL6 T0	IL6 T2	IL-1β T0	IL-1β T2	IL6+ IL-1β T0	IL6+ IL-1β T2
<65	11,93	9,39	4,45	6,38	4,16	42,72	8,61	49,10
	±9,34	±10,61	±4,54	±12,77	±2,67	±57,41	±6,08	±59,64
≥65	6,57	4,91	8,57	5,23	6,69	39,64	15,26	44,86
	±6,81	±8,47	±5,71	±12,68	±4,83	±38,95	±7,95	±44,60

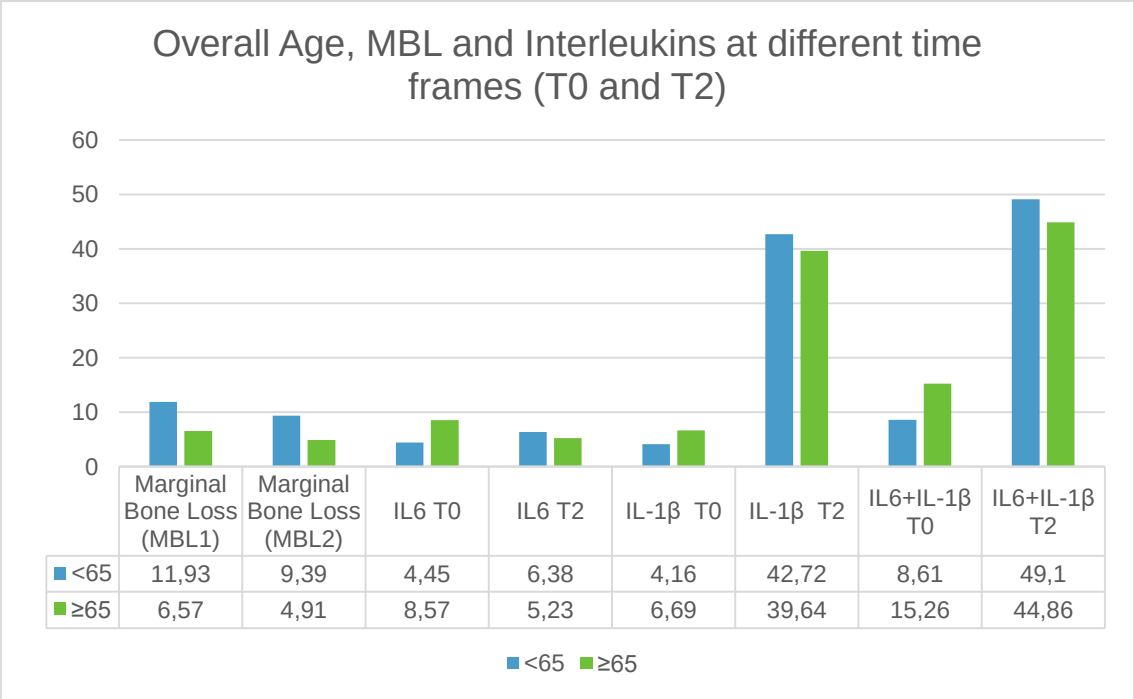


FIGURE 169 - Overall Values for Age, Marginal bone loss and time frames

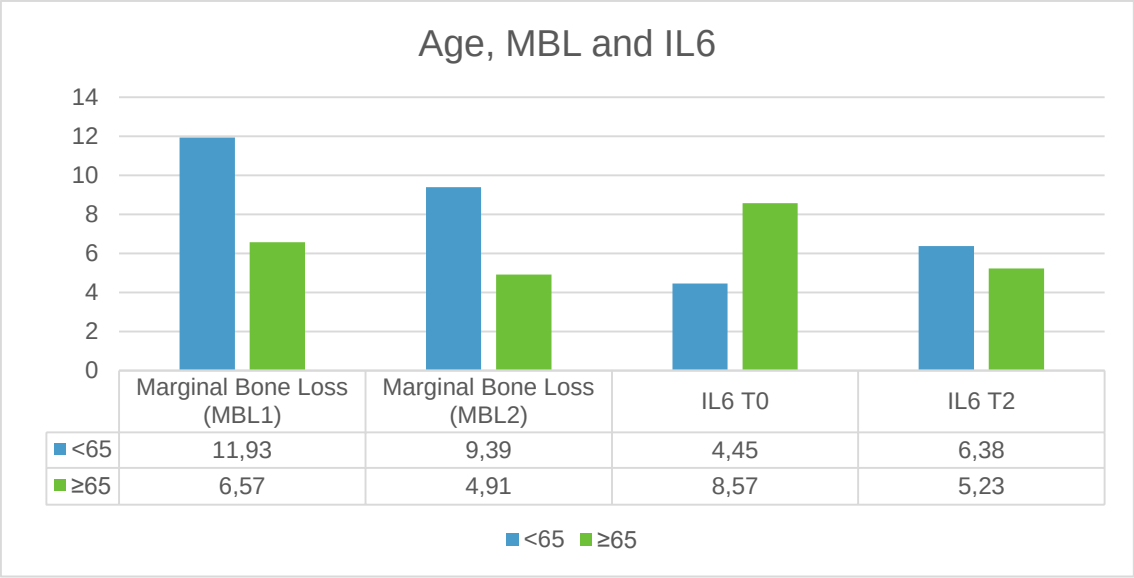


FIGURE 170 - Interleukin 6 behavior with age and correlation to marginal bone loss.

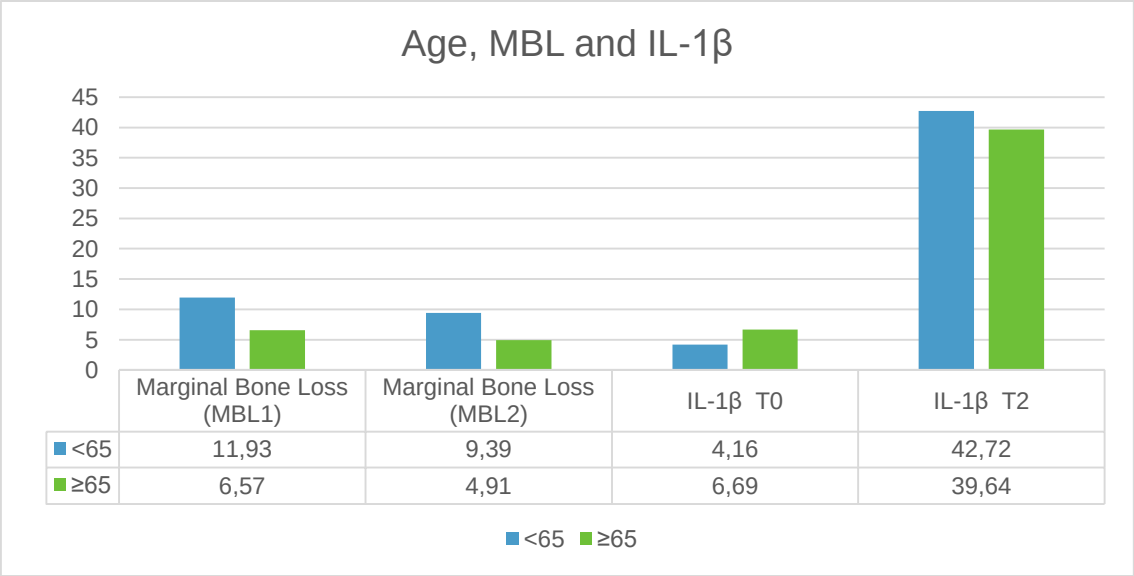


FIGURE 171 - Interleukin IL-1β behavior with age and correlation to marginal bone loss

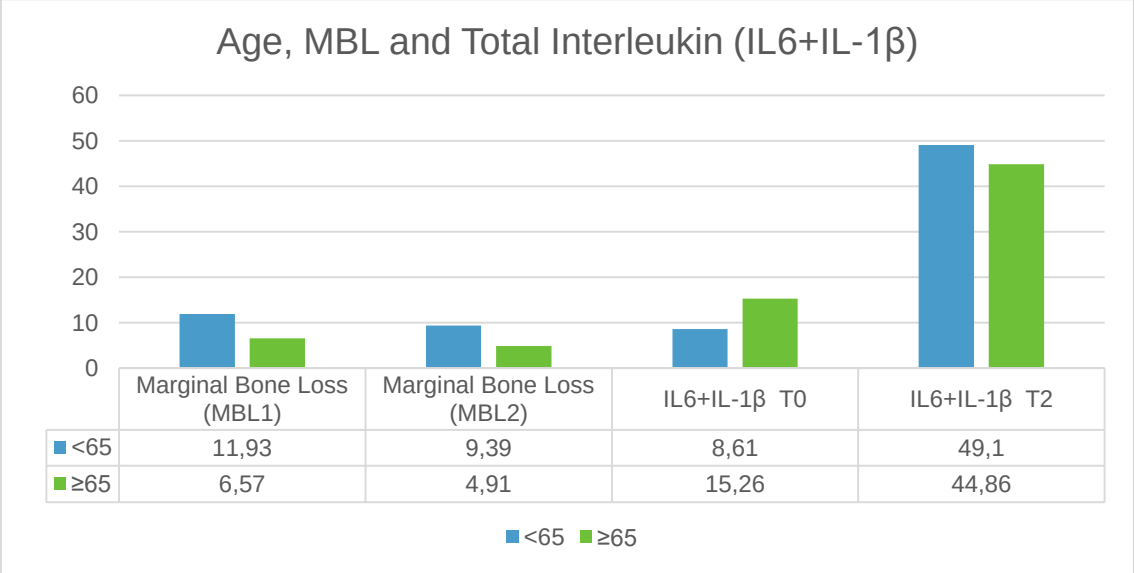


FIGURE 172 - Interleukin 6+ IL-1β behavior with age and correlation to marginal bone loss.

Correlation Between Age and Inflammatory Patterns

By correlating age to inflammatory reaction in relation to levels of IL-1β, IL6 and total IL6+ IL-1β different patterns of cytokine expression were observed.

In the final results, we found that at T2 (8 weeks), age did not significantly influence IL-1β (42 Vs 39 pg/ml – fig. 151), IL6 (6 Vs 5 pg/ml – fig. 150) and Total (49 Vs 44 pg/ml –fig. 152) values.

At T0, there was a different situation where IL6 differed significantly with age,

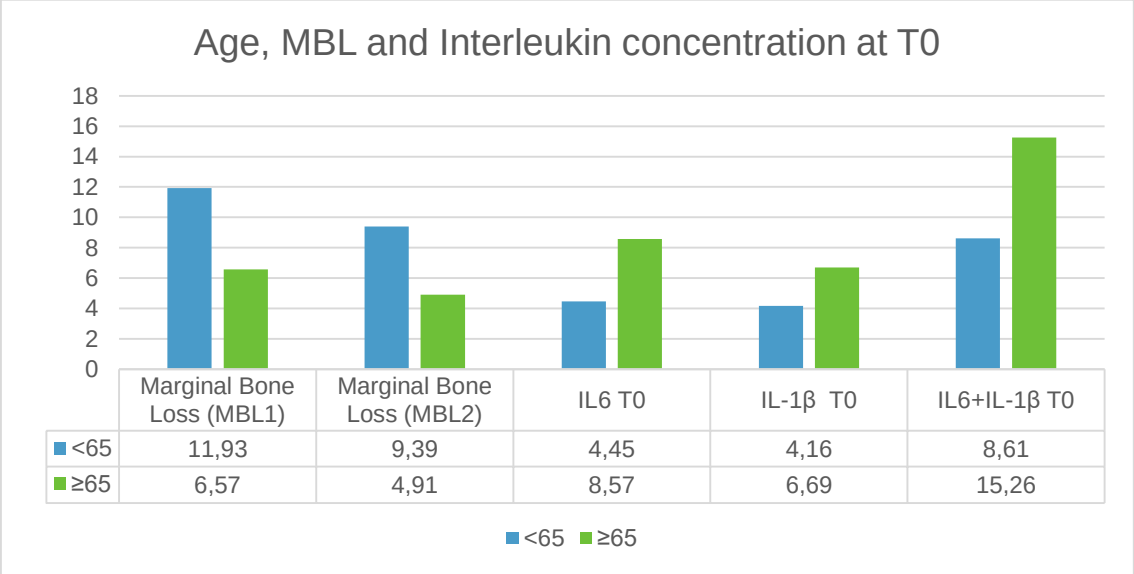
and, on average, IL6 was significantly higher at ≥65 years.

The same conclusions were drawn for IL-1β and in total (*p*-values = 0.047 and 0.002), showing that at T0 patients of 65 years or older tended to experience more inflammation (IL6, IL-1β and IL-1β+IL6) at early stages of implant placement than patients below 65 years.

Despite this, after 8 weeks Interleukin expression was the same in both groups.

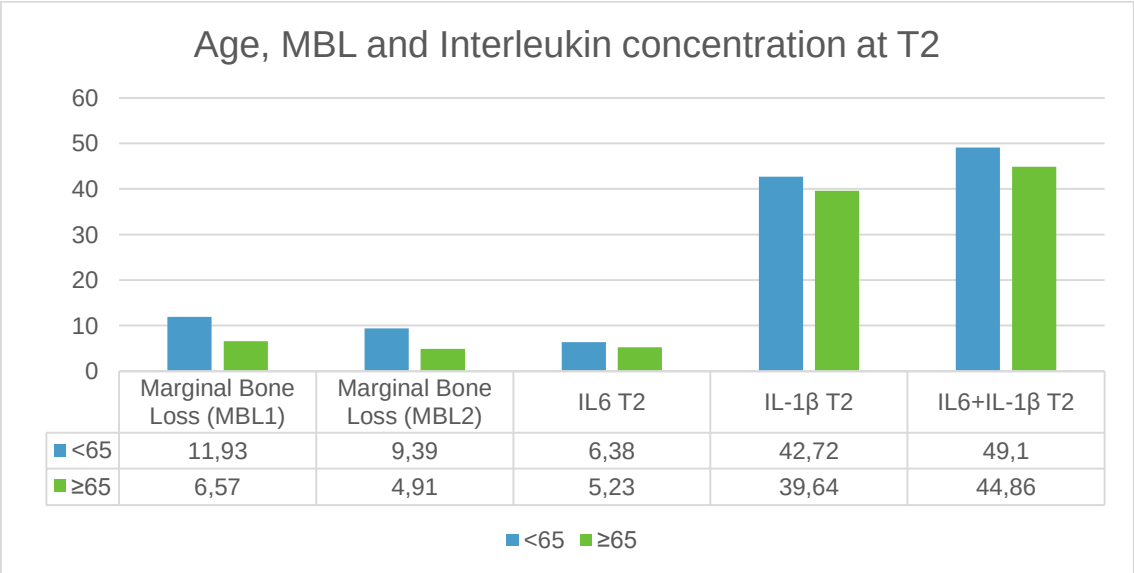
We can observe the differences in the boxplot charts in fig. 155,156 and 157.

The final statistic work can be found in tables 97-99.



	Marginal Bone Loss (MBL1)	Marginal Bone Loss (MBL2)	IL6 T0	IL-1β T0	IL6+IL-1β T0
<65	11,93	9,39	4,45	4,16	8,61
≥65	6,57	4,91	8,57	6,69	15,26

FIGURE 173 - Overall Interleukin behavior at T0 and correlation with marginal bone loss



	Marginal Bone Loss (MBL1)	Marginal Bone Loss (MBL2)	IL6 T2	IL-1β T2	IL6+IL-1β T2
<65	11,93	9,39	6,38	42,72	49,1
≥65	6,57	4,91	5,23	39,64	44,86

FIGURE 174 - Overall Interleukin behavior at T2 and correlation with marginal bone loss

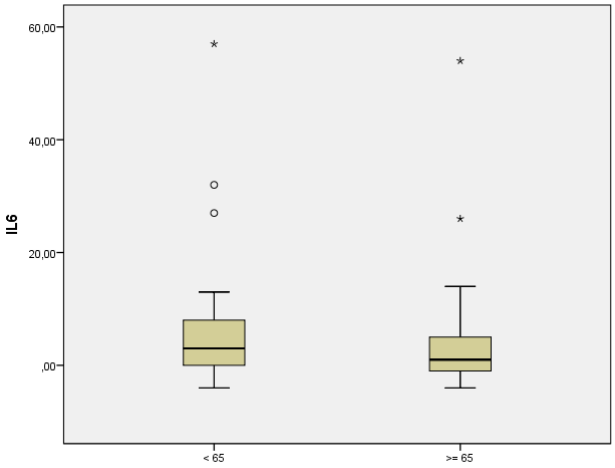


FIGURE 175 - Boxplot showing the interquartile differences of IL6 variation with age

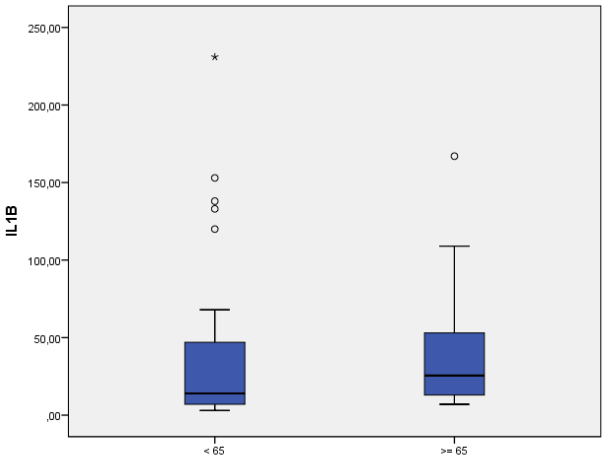


FIGURE 176 - Boxplot showing the interquartile differences of IL-1β variation with age

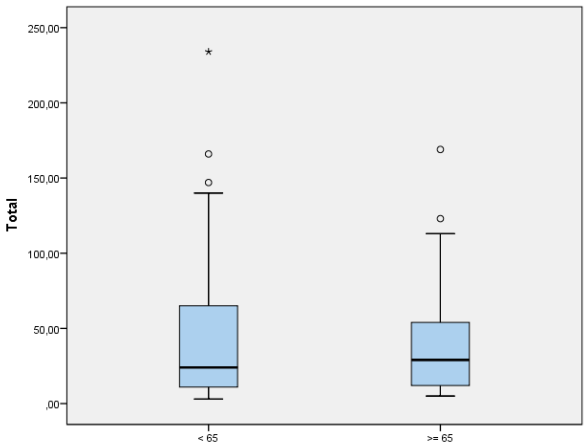


FIGURE 177 - Boxplot showing the interquartile differences of IL6+IL-1β variation with age

Table 97 - Correlations between MBL (MBL1 and MBL2) and Age (<65 /»65)

MBL	Test	P-Value	Correlation
MBL1 with Age (<65 /»65)	Mann-Whitney	0,020	Yes
MBL2 with Age (<65 /»65)	Mann-Whitney	0,074	No

Table 98 - Correlations between Interleukin Levels and Age (<65 /»65) at T0

Interleukin (IL)	Test	P-Value	Correlation
IL-1β with Age at T0	Mann-Whitney	0,047	Yes
IL6 with Age at T0	T-Test	0,005	Yes
IL6+IL1β with Age at T0	Mann-Whitney	0,002	Yes

Table 99 - Correlations between Interleukin Levels and Age (<65 /»65) at T2

Interleukin (IL)	Test	*P*-Value	Correlation
IL-1β with Age at T2	Mann-Whitney	0,220	No
IL6 with Age at T2	Mann-Whitney	0,414	No
IL6+ IL-1β with Age at T2	Mann-Whitney	0,568	No

SECTION 5.7. SECONDARY OUTCOME MEASURES: ZIRCONIA, ACRYLIC AND TITANIUM INFLAMMATION LEVELS OF IL6 AND IL-1β AND CORRELATION TO MARGINAL BONE LOSS AND GENDER- HYPOTHESIS AND RESULTS

Section 5.7.1. Hypothesis

Correlation between Gender, marginal bone loss and inflammation

With Gender as the central variable and independent of the abutment material placed two groups were considered: male (ML) and female (FM).

To relate Gender and Marginal Bone loss, independent of the material

Specific aim 1- to relate gender to Marginal Bone Loss (MBL1) and (MBL2) Overall at T1 (8 weeks).

H0: There is no difference between gender in the two given groups and marginal bone loss in implants placed under the standard protocol.

H1 There is a difference between gender in the two given groups and marginal bone loss in implants placed under the standard protocol.

Relate Gender and Inflammatory Levels

Specific aim 2: To relate gender to Overall Inflammatory Levels at T1 (8 weeks).

H0: There is no difference between gender in the two given groups and overall inflammatory levels (IL-1β +IL6) in implants placed under the standard protocol.

H1: There is a difference between gender in the two given groups and overall inflammatory levels (IL-1β +IL6) in implants placed under the standard protocol.

Specific aim 3: Relate with Inflammatory Levels Interleukin IL-1β at T2 (8 weeks)

H0: There is no difference between gender in the two given groups and inflammatory levels (IL-1β) in implants placed under the standard protocol.

H1: There is a difference between gender in the two given groups and inflammatory levels (IL-1β) in implants placed under the standard protocol.

Specific aim 4: To relate gender to Interleukin IL6 Inflammatory Levels at T2 (8 weeks)

H0: There is no difference between gender in the two given groups and inflammatory levels (IL6) in implants placed under the standard protocol.

H1: There is a difference between gender in the two given groups and inflammatory levels (IL6) in implants placed under the standard protocol.

Specific aim 5: To relate gender to Overall Inflammatory Levels at T0 (Baseline)

H0: There is no difference between gender in the two given groups and overall inflammatory levels (IL-1β +IL6) in implants placed under the standard protocol.

H1: There is a difference between gender in the two given groups and overall inflammatory levels (IL-1β +IL6) in implants placed under the standard protocol.

Specific aim 6: To relate gender to Interleukin IL-1β Inflammatory Levels at T0 (Baseline)

H0: There is no difference between gender in the two given groups and inflammatory levels (IL-1β) in implants placed under the standard protocol.

H1: There is a difference between gender in the two given groups and inflammatory levels (IL-1β) in implants placed under the standard protocol.

Specific aim 7; To relate gender to Interleukin IL6 Inflammatory Levels at T0 (Baseline)

H0: There is no difference between gender in the two given groups and inflammatory levels (IL6) in implants placed under the standard protocol.

H1: There is a difference between gender in the two given groups and

inflammatory levels (IL6) in implants placed under the standard protocol.

Section 5.7.2. Results

In this chapter, we wanted to correlate inflammatory levels with Gender and marginal bone loss (MBL1 and MBL2). Table 100 shows the raw data collected, divided by age and material used.

Table 100 - Mean average of concentration of Interleukins and Gender

Implant #	Tooth #	Material	Gender
1	47	A	F
2	45	A	M
3	25	A	M
4	16	Z	M
5	36	T	F
6	25	T	M
7	37	T	M
8	37	A	M
9	46	A	F
10	17	T	F
11	24	A	F
12	22	A	M
13	36	Z	M
14	26	T	M
15	46	Z	M
16	11	Z	F
17	25	T	M
18	36	Z	M

19	15	Z	M
20	37	T	F
21	46	Z	M
22	16	A	M
23	26	T	M
24	14	Z	F
25	14	Z	M
26	24	T	F
27	25	A	M
28	15	T	M
29	36	Z	F
30	36	A	M
31	24	T	M
32	46	T	M
33	46	Z	M
34	16	Z	M
35	24	T	F
36	45	A	M
37	26	T	M
38	24	Z	M
39	16	A	M
40	25	Z	F
41	15	Z	M
42	23	Z	F
43	14	Z	M
44	14	A	M
45	36	A	F
46	25	Z	M
47	24	A	M

48	35	T	M
49	45	T	M
50	15	A	F
51	44	T	F
52	24	A	F
53	25	T	F
54	26	Z	M
55	25	Z	M
56	46	Z	M
57	15	A	F
58	35	A	F
59	12	Z	F
60	34	A	F

The collected data was arranged into average DS and SD. In table 100, the mean average of MBL and IL concentrations by gender are shown.

Table 101 - Mean average of concentration of Interleukins and Gender

Gender	MBL1	MBL2	IL6 T0	IL6 T2	IL-1β T0	IL-1β T2	IL6+ IL-1β T0	IL6+ IL-1β T2
M	7,7 ±6,29	4,59 ±6,99	7,45 ±5,85	7,23 ±14,72	5,5 ±3,60	41,09 ±42,52	12,97 ±7,87	48,32 ±47,48
F	13,20 ±11,10	12,57 ±12,17	4,36 ±4,23	3,8 ±8,31	4,86 ±4,39	41,85 ±60,70	9,23 ±6,82	45,65 ±62,35

With regard to gender, there was once again an uneven sample distribution, with 36 males and 22 females for sample reading. (fig. 158).

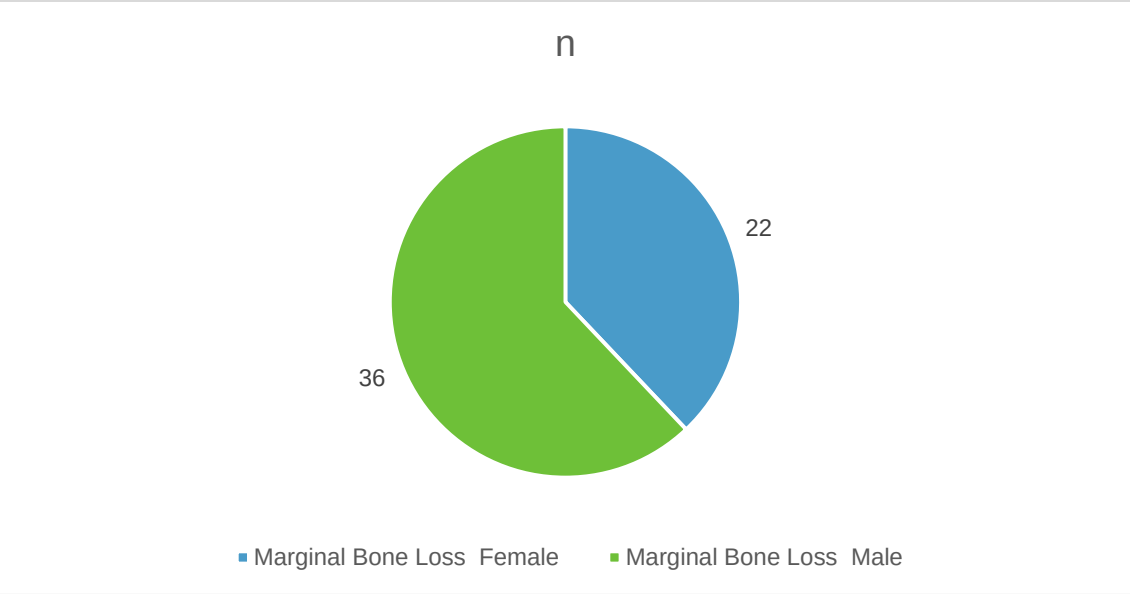


FIGURE 178 - Sample size for correlation between gender, marginal bone loss

Correlation Between Gender and Marginal Bone loss

When comparing Gender with marginal bone loss, there is a tendency for the female gender to lose more bone than males.

There are no statistically significant differences between marginal bone loss in males when compared to females (7,7 mm Vs 13,2 mm) when we considered all measures in MBL1 (table 102).

However, if we look at MBL2 the differences are statistically significantly different (4,59mm Vs 12,57) being higher in the female gender, showing that in the female group presented at T2 there were more implants with marginal bone loss apical to the implant platform than males, (*p*-value = 0,043) (table 102).

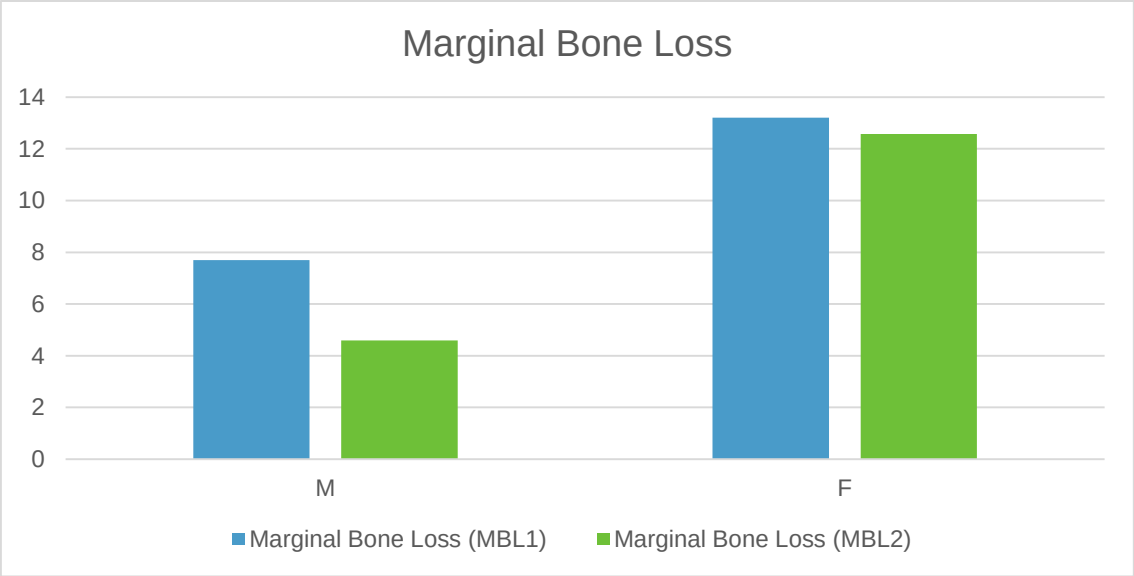
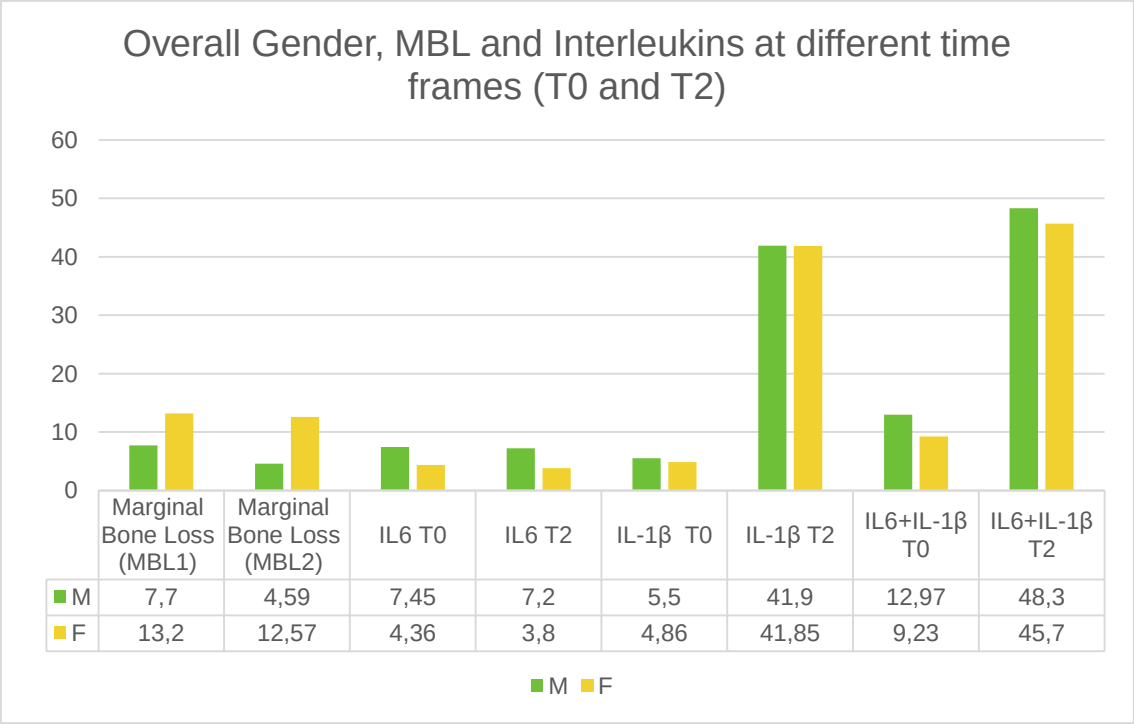


FIGURE 179 - Marginal bone Loss (MBL1 and MBL2) found in the two different genders, note the higher tendency for resorption in the female gender.



	Marginal Bone Loss (MBL1)	Marginal Bone Loss (MBL2)	IL6 T0	IL6 T2	IL-1β T0	IL-1β T2	IL6+IL-1β T0	IL6+IL-1β T2
M	7,7	4,59	7,45	7,2	5,5	41,9	12,97	48,3
F	13,2	12,57	4,36	3,8	4,86	41,85	9,23	45,7

FIGURE 180 - Overall gender inflammatory pattern variation at each time frame (T0 to T2)

Correlation Between Gender and Inflammatory Patterns

In relation to the comparison of gender with IL levels at T0, the results are very simple for IL-1β (5,5 vs 4,86 pg/ml- fig.152) showinging that there were no differences in terms of gender.

Again, at T0, IL6 (fig. 161) exhibited a different behavior to IL-1β, where IL6

was, on average, significantly higher in males (*p*-value = 0,038) (table 103)

With regard to inflammatory indicators at T2, indicators IL-1β, IL6 and total (IL-1β +IL6) did not differ significantly with gender.

The other indicators did not differ significantly with gender (table 104).

The boxplots showing IL variation are presented in fig. 166-168.

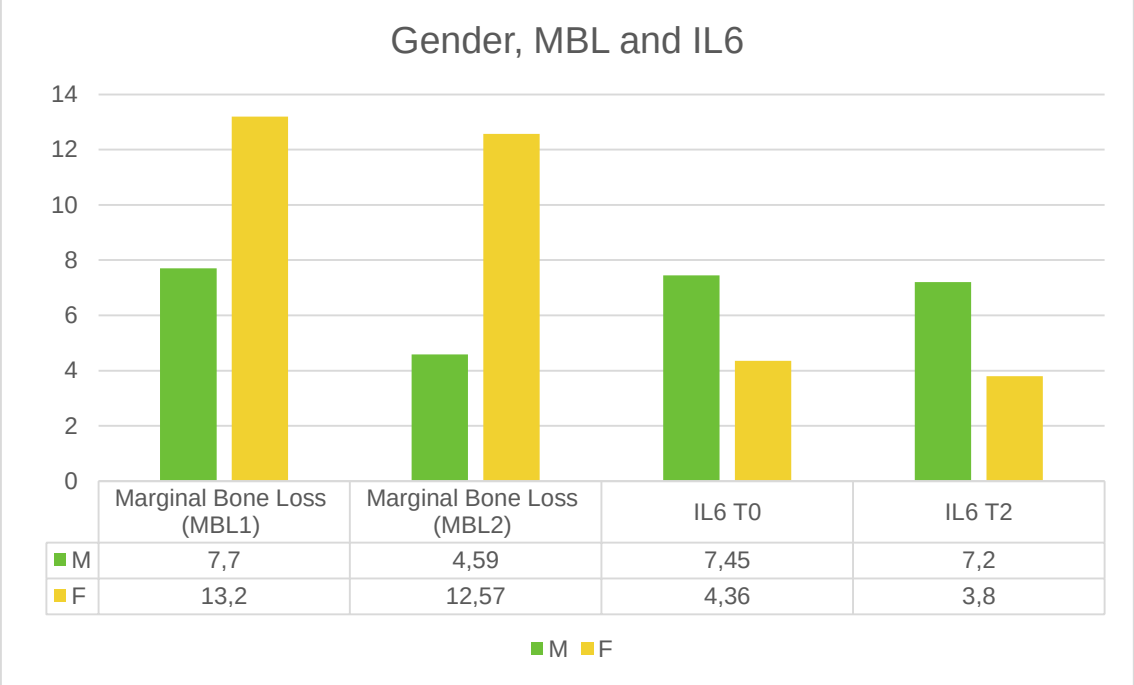


FIGURE 181 - Overall results and inflammatory pattern of IL6 by time frame and Gender. Marginal bone loss comparison in the two genders (M vs F)

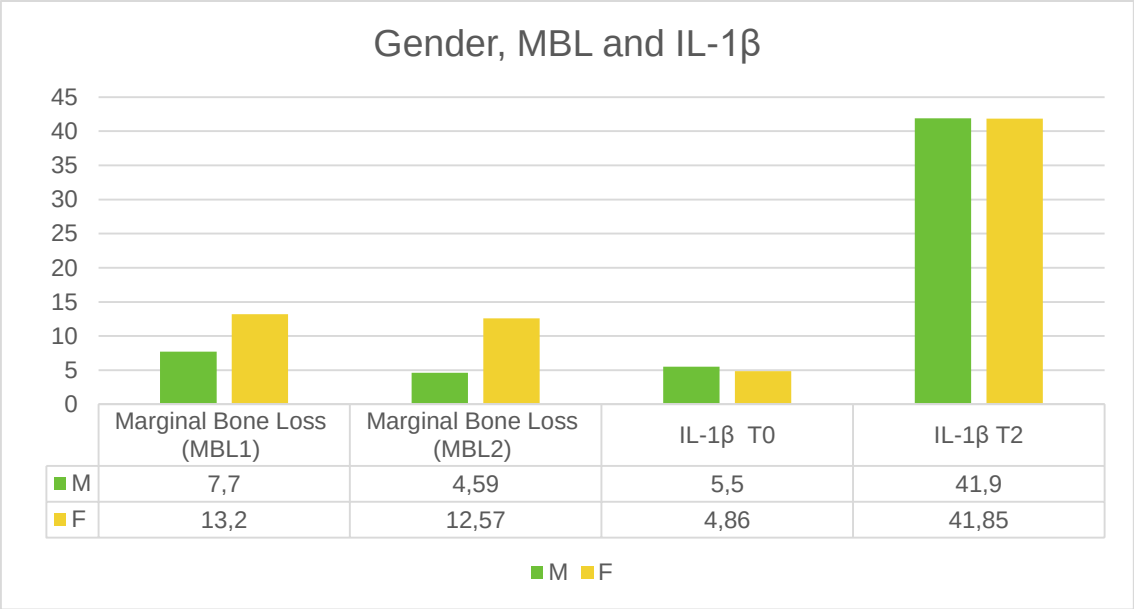


FIGURE 182 - Overall results and inflammatory pattern of IL-1β by time frame and Gender. Marginal bone loss comparison in the two genders (ML vs FM)

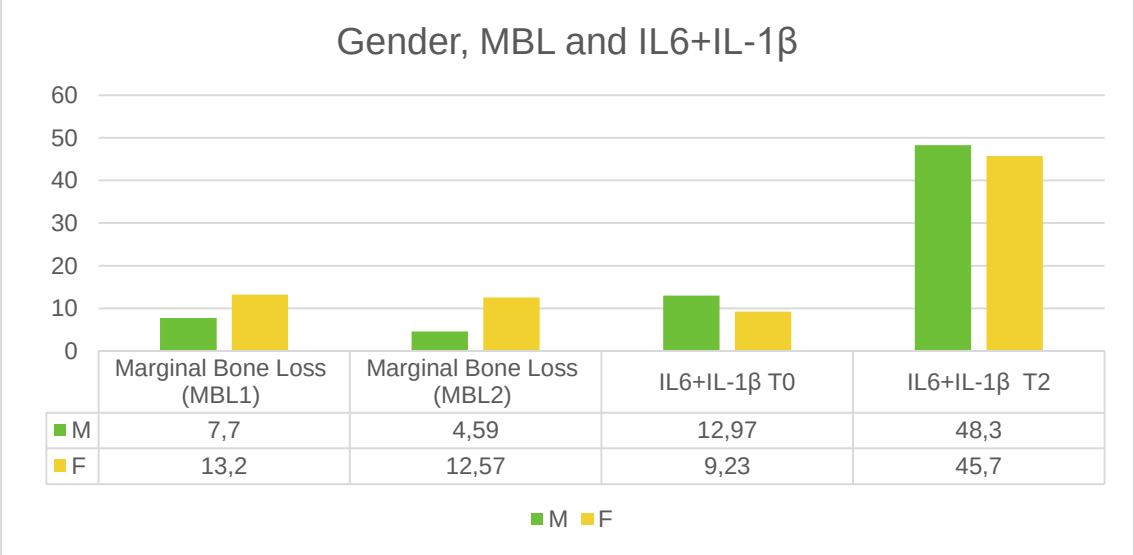


FIGURE 183 - Overall results and inflammatory pattern of IL-1β +IL6 by time frame and Gender. Marginal bone loss comparison in the two genders (ML vs FM).

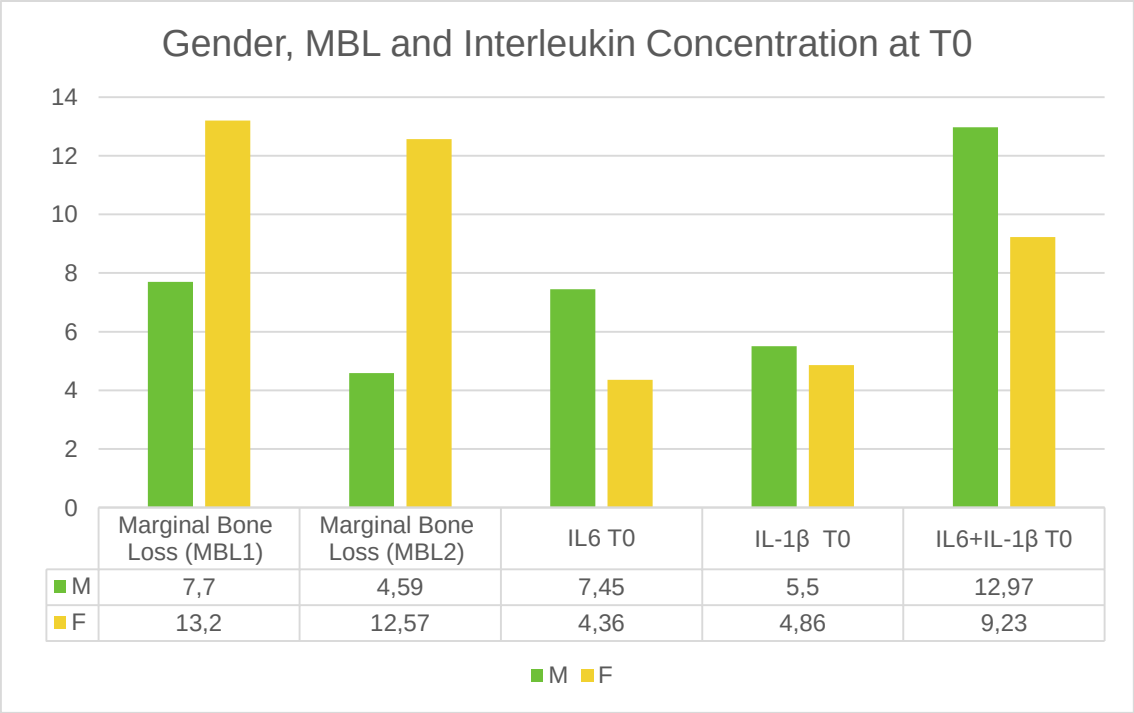


FIGURE 184 - Overall results and inflammatory pattern by time frame (baseline T0) and Gender. Marginal bone loss comparison in the two genders (ML vs FM).

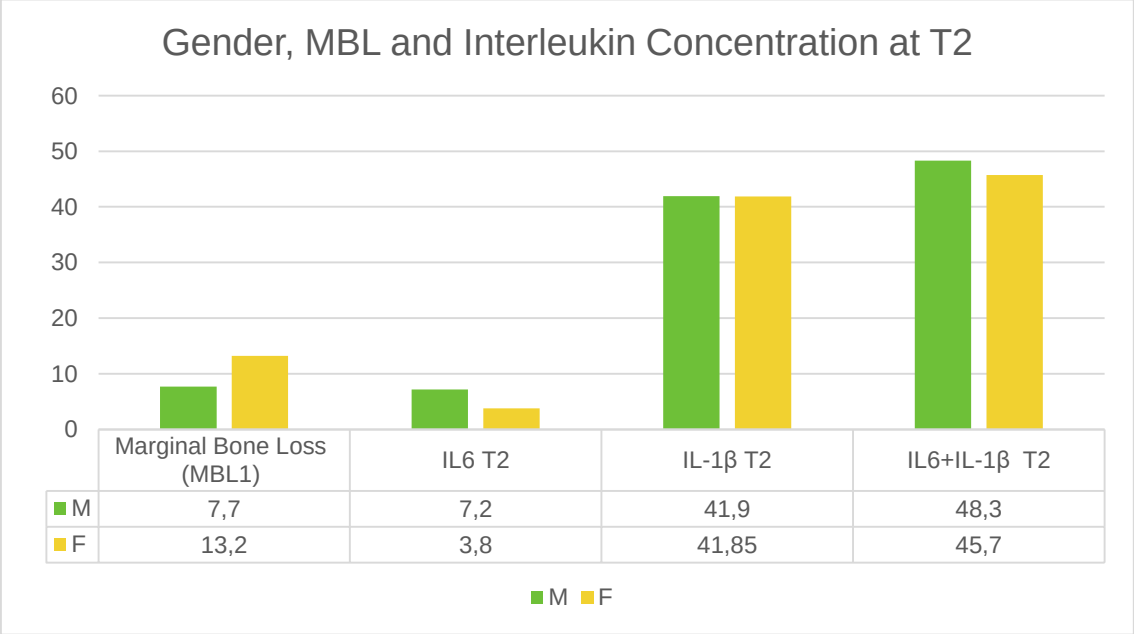


FIGURE 185 - Overall results and inflammatory pattern by time frame (baseline T2) and Gender. Marginal bone loss comparison in the two genders (ML vs FM).

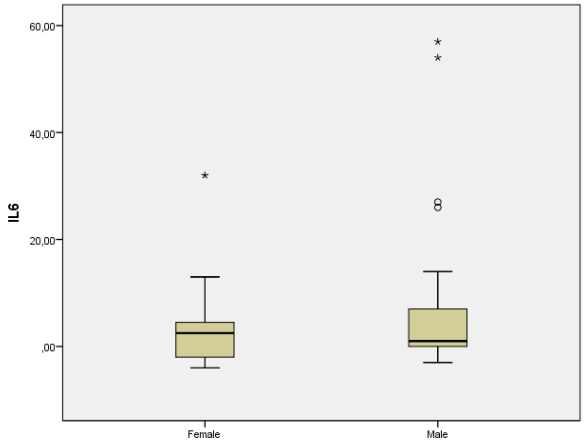


FIGURE 186 - Boxplot showing the interquartile differences of IL6 variation with gender.

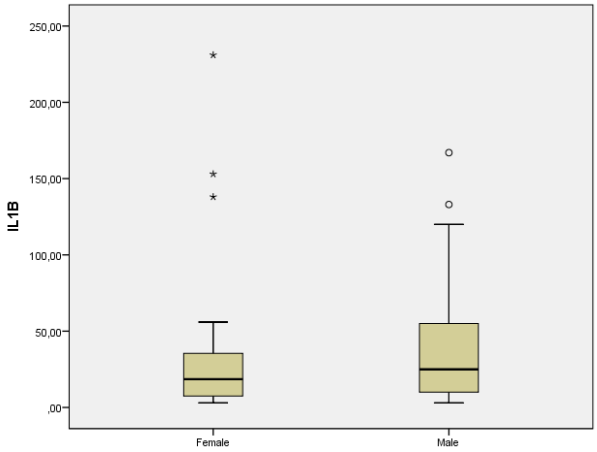


FIGURE 187 - Boxplot showing the interquartile differences of IL-1β variation with gender.

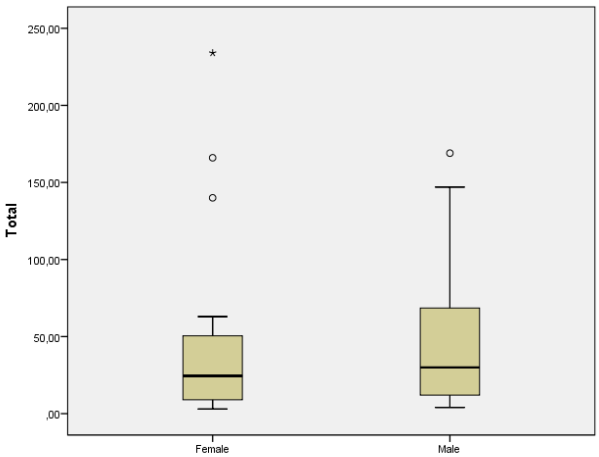


FIGURE 188 - Boxplot showing the interquartile differences of IL6+IL-1β variation with gender.

Table 102 - Correlations between MBL (MBL1 and MBL2) and Gender (Male/Female)

MBL	Test	P-Value	Correlation
MBL1 with Gender	Mann-Whitney	0,142	No
MBL2 with Gender	Mann-Whitney	0,005	Yes

Table 103 - Correlations between Interleukin Levels and Gender (Male/Female)

Interleukin (IL)	Test	*P*-Value	Correlation
IL-1β with Gender at T0	Mann-Whitney	0,180	No
IL6 with Gender at T0	T-Test	0,038	Yes
IL6+IL1β Gender at T0	Mann-Whitney	0,105	No

Table 104 - Correlations between Interleukin Levels and Gender (Male/Female)

Interleukin (IL)	Test	*P*-Value	Correlation
IL-1β with Gender at T2	Mann-Whitney	0,505	No
IL6 with Gender at T2	Mann-Whitney	0,330	No
IL6+IL-1β Gender at T2	Mann-Whitney	0,751	No

SECTION 5.8. SECONDARY OUTCOME MEASURES: ZIRCONIA, ACRYLIC AND TITANIUM INFLAMMATION LEVELS OF IL6 AND I IL-1β AND CORRELATION TO MARGINAL BONE LOSS (MBL) AND ANATOMICAL POSITION (MAXILLA VS MANDIBLE)- HYPOTHESIS AND RESULT

Section 5.8.1. Hypothesis

Correlation between Anatomical position (Maxilla vs Mandible), marginal bone loss (MBL1 and MBL2) and inflammation.

With Anatomical Position as the central variable and independent of the abutment material placed two groups were considered: Maxilla and Mandible.

To relate Anatomical Position **to Marginal Bone loss, independent of the material.**

Specific aim 1: To relate anatomical position to Overall Marginal Bone Loss (MBL1 and MBL2) at T2 (8 weeks)

H0: There is no difference between Anatomical Position (in the two given groups) and marginal bone loss in implants placed under the standard protocol.

H1: There is a difference between Anatomical Position (in the two given groups) and marginal bone loss in implants placed under the standard protocol.

To relate Anatomical Position and Inflammatory Levels.

Specific aim 2: To relate anatomical position to Overall Inflammatory Levels at T2 (8 weeks)

H0: There is no difference between Anatomical Position (in the two given groups) and overall inflammatory levels (IL-1β+IL6) in implants placed under the standard protocol.

H1: There is a difference between Anatomical Position (in the two given groups) and overall inflammatory levels (IL-1β+IL6) in implants placed under the standard protocol.

Specific aim 3: To relate anatomical position to Interleukin IL-1β Inflammatory Levels at T2 (8 weeks)

H0: There is no difference between Anatomical Position (in the two given groups) and inflammatory levels (IL-1β) in implants placed under the standard protocol.

H1: There is a difference between Anatomical Position (in the two given groups) and inflammatory levels (IL-1β) in implants placed under the standard protocol.

Specific aim 4: To relate anatomical position to Interleukin IL6 Inflammatory Levels at T2 (8 weeks)

H0: There is no difference between Anatomical Position (in the two given groups) and inflammatory levels (IL6) in implants placed under the standard protocol.

H1: There is a difference between Anatomical Position (in the two given groups) and inflammatory levels (IL6) in implants placed under the standard protocol.

Specific aim 5: To relate anatomical position to Overall Inflammatory Levels at T0 (Baseline)

H0: There is no difference between Anatomical Position (in the two given groups) and overall inflammatory levels (IL-1β+IL6) in implants placed under the standard protocol.

H1: There is a difference between Anatomical Position (in the two given groups) and overall inflammatory levels (IL-1β+IL6) in implants placed under the standard protocol.

Specific aim 6: To relate anatomical position to Interleukin IL-1β Inflammatory Levels at T0 (Baseline).

H0: There is no difference between Anatomical Position (in the two given groups) and inflammatory levels (IL-1β) in implants placed under the standard protocol.

H1: There is a difference between Anatomical Position (in the two given groups) and inflammatory levels (IL-1β) in implants placed under the standard protocol.

Specific aim 7: To relate anatomical postion to Interleukin IL6 Inflammatory Levels at T0 (Baseline).

H0: There is no difference between Anatomical Position (in the two given groups) and inflammatory levels (IL6) in implants placed under the standard protocol.

H1: There is a difference between Anatomical Position (in the two given groups) and inflammatory levels (IL6) in implants placed under the standard protocol.

Section 5.8.2. Results

In this chapter, we wanted to correlate inflammatory levels to anatomical position (maxilla/mandible) and marginal bone loss (MBL1 and MBL2). Table 105 shows the raw data collected divided by position and material used.

Table 105 - Collected data relating implant position (maxilla vs mandible) and the type of healing abutment placed

Implant #	Tooth #	Material
1	47	A
2	45	A
3	25	A
4	16	Z

5	36	T
6	25	T
7	37	T
8	37	A
9	46	A
10	17	T
11	24	A
12	22	A
13	36	Z
14	26	T
15	46	Z
16	11	Z
17	25	T
18	36	Z
19	15	Z
20	37	T
21	46	Z
22	16	A
23	26	T
24	14	Z
25	14	Z
26	24	T
27	25	A
28	15	T
29	36	Z
30	36	A
31	24	T
32	46	T
33	46	Z

34	16	Z
35	24	T
36	45	A
37	26	T
38	24	Z
39	16	A
40	25	Z
41	15	Z
42	23	Z
43	14	Z
44	14	A
45	36	A
46	25	Z
47	24	A
48	35	T
49	45	T
50	15	A
51	44	T
52	24	A
53	25	T
54	26	Z
55	25	Z
56	46	Z
57	15	A
58	35	A
59	12	Z
60	34	A

There were 23 samples for the maxilla and 35 for the mandible as displayed in figure 169.

Final results by anatomical position are displayed in table 106.

Table 106 - Mean average and standard deviation for Interleukins and time frame, and marginal bone loss related to anatomical position (maxilla vs mandible)

Anatomical Position	MBL1	MBL2	IL6 T0	IL6 T2	IL-1β T0	IL-1β T2	IL6+IL-1β T0	IL6+IL-1β T2
Max	9,17 ±9,85	5,92 ±10,83	5,79 ±4,83	4,48 ±11,98	4,97 ±3,94	46,19 ±55,85	10,76 ±7,71	50,68 ±58,49
Man	10,77 ±6,91	10,19 ±8,10	6,86 ±6,33	8,05 ±13,57	5,67 ±3,93	33,95 ±38,99	12,52 ±7,54	42 ±44,77

Max- Maxilla Mand- Mandible MBL1 - Marginal Bone Loss MBL2- Marginal Bone Loss (only implant exposed in mm)

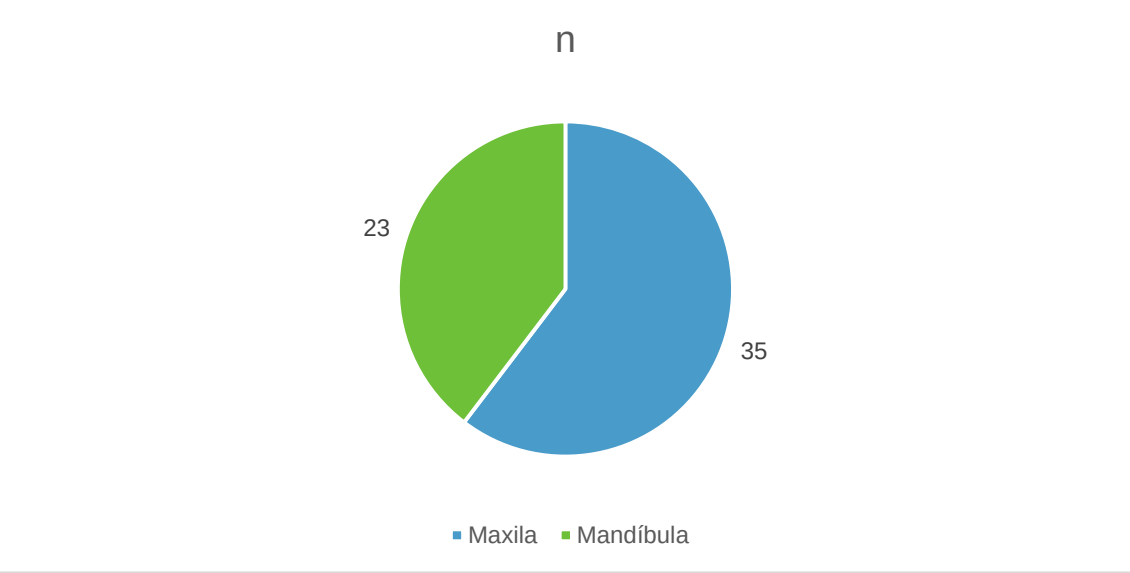
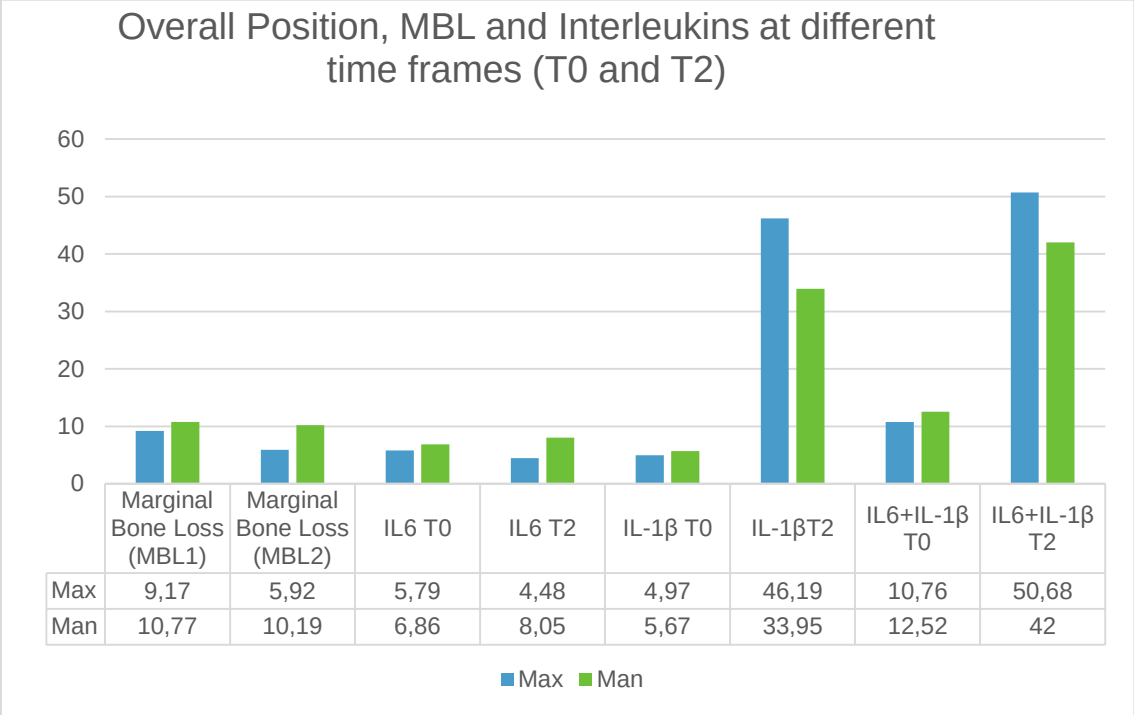


FIGURE 189 - Sample size for correlation between anatomical position, marginal bone loss and inflammation

Correlation Between Anatomical Position and Marginal Bone Resorption

In order o correlate the 3 items; MBL, inflammation and biomaterials with the final results we found out that if we compared only MBL1 to anatomical position there were no statistical differences between maxilla and mandible (9,17 mm Vs

10,77mm) (fig. 170). However, if we compared MBL2 to anatomical position then bone resorption on the maxilla vs mandible, differed significantly with the position (*p*-value = 0,023 table 107) where it was higher in the mandible (5,92 Vs 10,19). This show that there were more implants with marginal bone loss apical to the implant platform on the mandible than on the maxilla.



	Marginal Bone Loss (MBL1)	Marginal Bone Loss (MBL2)	IL6 T0	IL6 T2	IL-1β T0	IL-1βT2	IL6+IL-1β T0	IL6+IL-1β T2
Max	9,17	5,92	5,79	4,48	4,97	46,19	10,76	50,68
Man	10,77	10,19	6,86	8,05	5,67	33,95	12,52	42

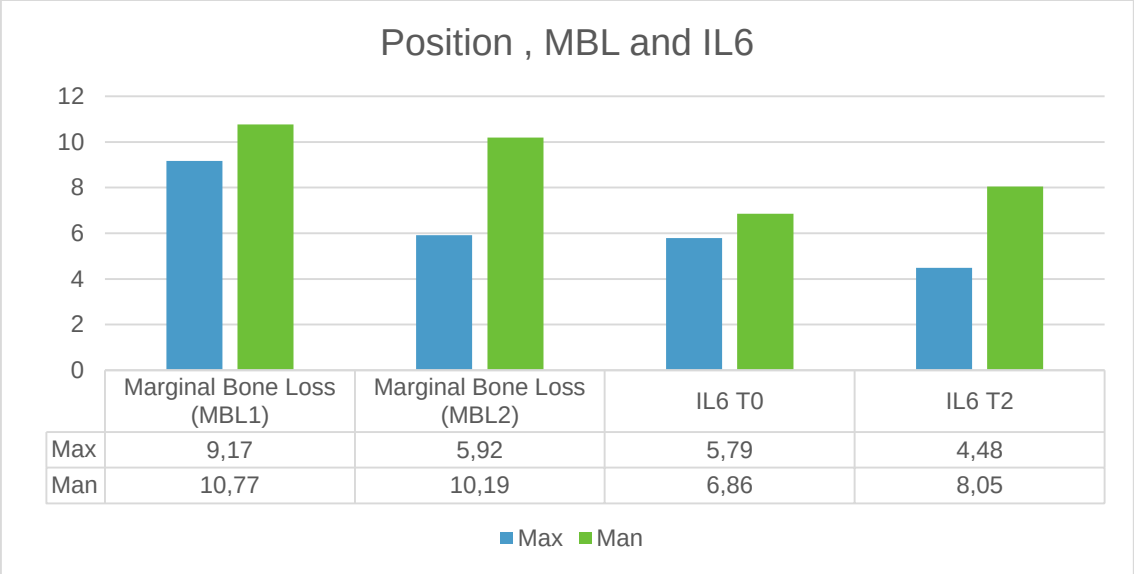
FIGURE 190 - Overall marginal bone loss and inflammatory variation at different time frames between maxilla and mandible

Correlation Between Anatomical Position and Inflammatory Patterns

The inflammatory patterns were very similar between the maxilla and mandible both at T0 and at T2.

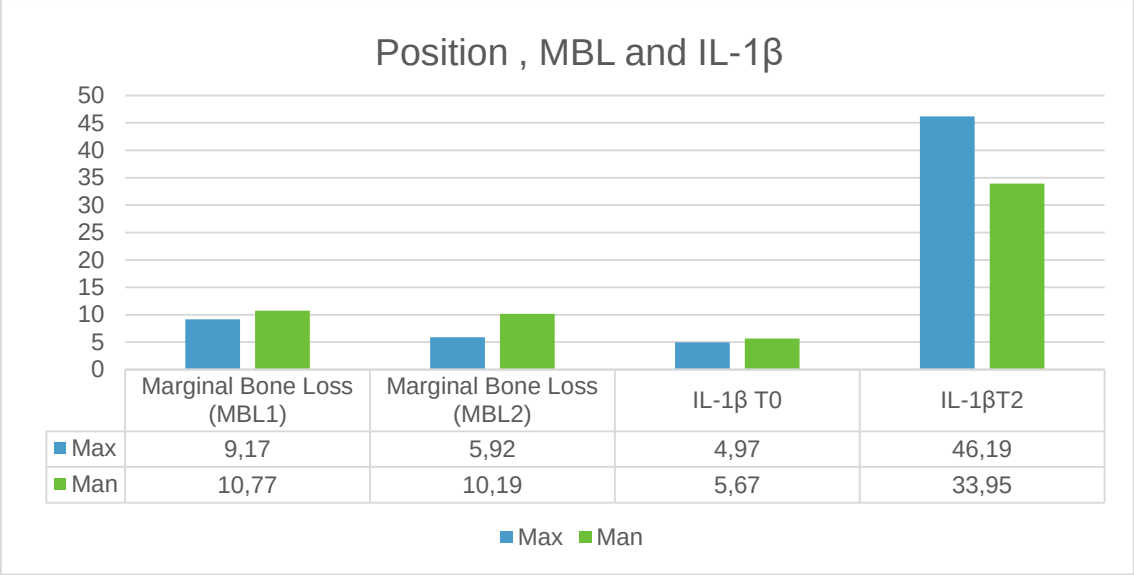
None of the 3 inflammatory indicators (IL6, IL-1β and total IL6 + IL-1β) at T2 differed significantly with the position. The same conclusion can be drawn for T0 (baseline).

The statistical tests for significance of inflammatory reaction between arcades in table 108 and 109.



	Marginal Bone Loss (MBL1)	Marginal Bone Loss (MBL2)	IL6 T0	IL6 T2
Max	9,17	5,92	5,79	4,48
Man	10,77	10,19	6,86	8,05

FIGURE 191 - Overall marginal bone loss and inflammatory variation of IL6, between maxilla and mandible.



	Marginal Bone Loss (MBL1)	Marginal Bone Loss (MBL2)	IL-1β T0	IL-1βT2
Max	9,17	5,92	4,97	46,19
Man	10,77	10,19	5,67	33,95

FIGURE 192 - Overall marginal bone loss and inflammatory variation of IL-1β, between maxilla and mandible.

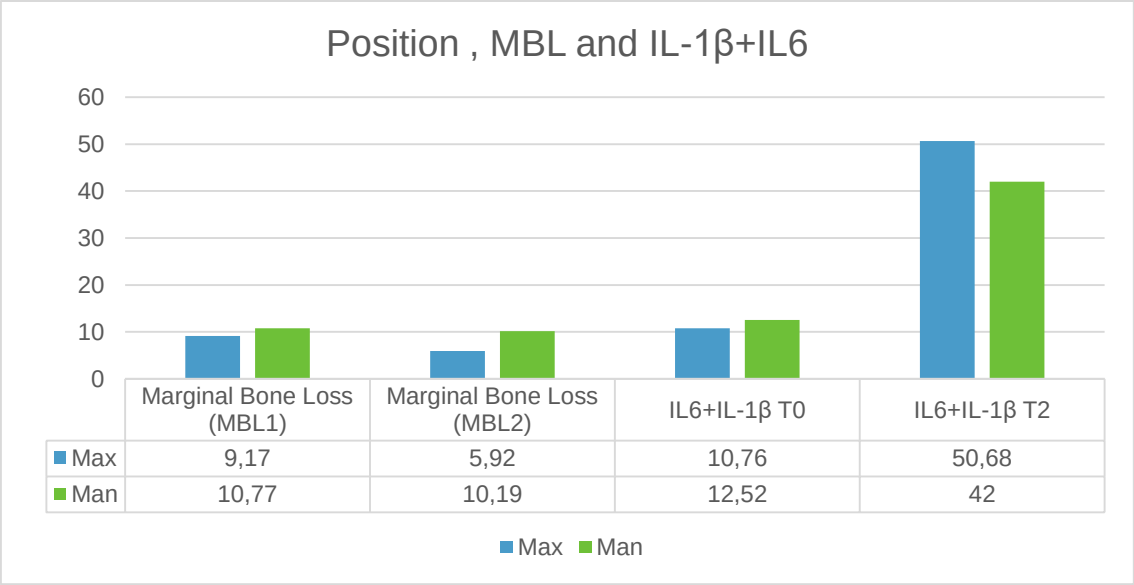


FIGURE 193 - Overall marginal bone loss and inflammatory variation of IL6+IL-1β, between maxilla and mandible.

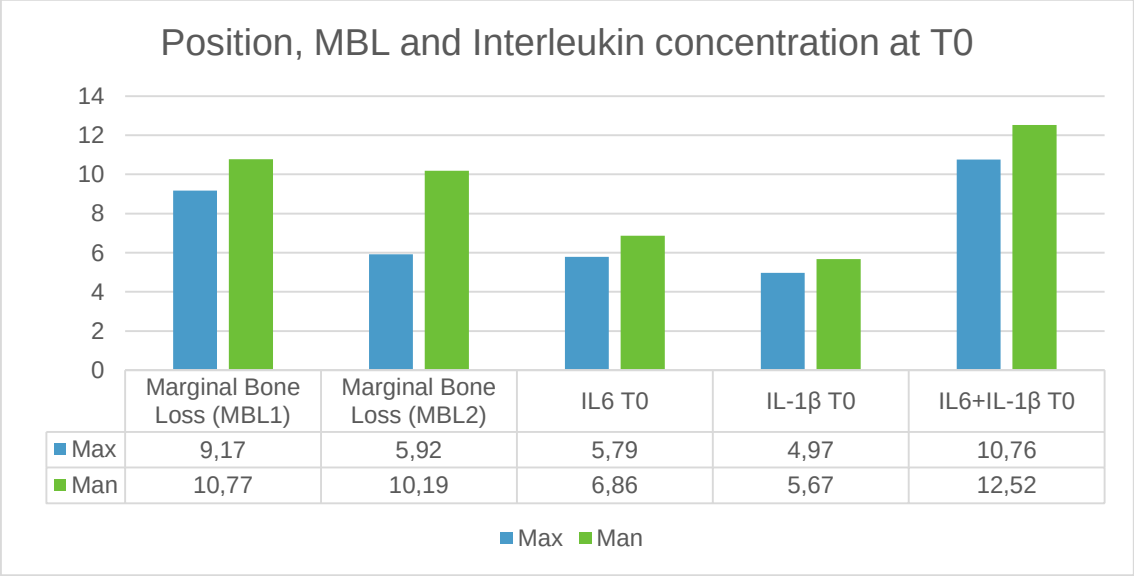
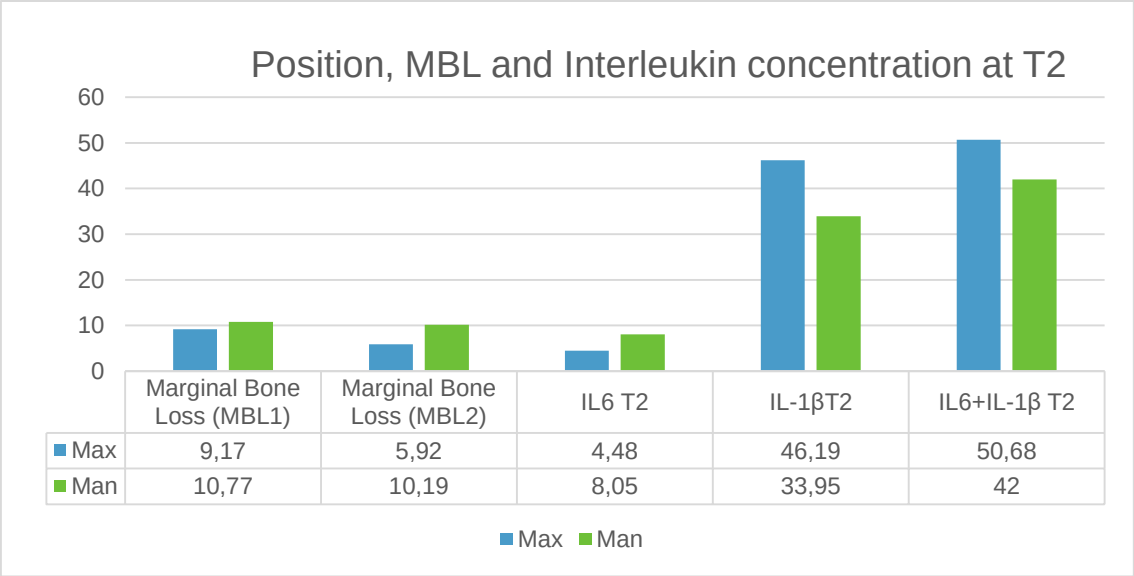


FIGURE 194 - Overall marginal bone loss and inflammatory variation of IL at baseline T0, between maxilla and mandible.



	Marginal Bone Loss (MBL1)	Marginal Bone Loss (MBL2)	IL6 T2	IL-1βT2	IL6+IL-1β T2
Max	9,17	5,92	4,48	46,19	50,68
Man	10,77	10,19	8,05	33,95	42

FIGURE 195 - Overall marginal bone loss and inflammatory variation of IL at baseline T2, between maxilla and mandible.

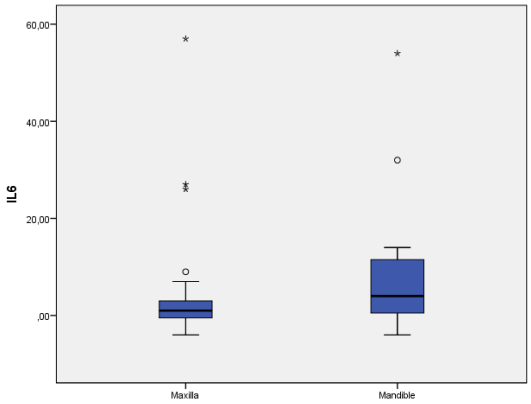


FIGURE 196 - Boxplot showing the interquartile differences of IL6 variation with anatomical position.

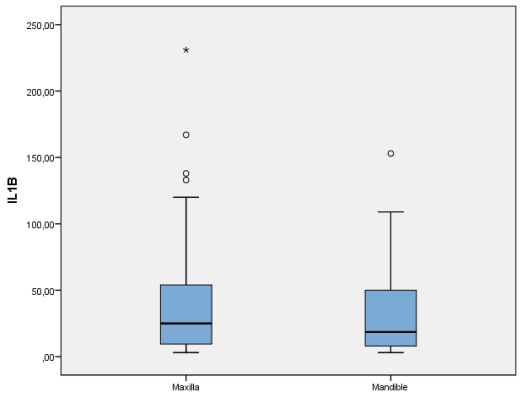


FIGURE 197 - Boxplot showing the interquartile differences of IL-1β variation with anatomical position.

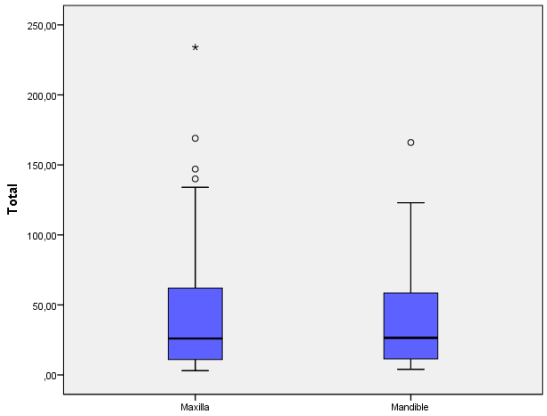


FIGURE 198 - Boxplot showing the interquartile differences of IL6+IL-1β variation with anatomical position.

Table 107 - Correlations between MBL (MBL1 and MBL2) and anatomical position (Maxilla/Mandible)

MBL	Test	P-Value	Correlation
MBL1 with **Position**	T-test	0,502	No
MBL2 with **Position**	T-test	0,023	Yes

Table 108 - Correlations between Interleukin Levels and anatomical position (Maxilla/Mandible at T0)

Interleukin (IL)	Test	*P*-Value	Correlation
IL-1β w/ **Position** at T0	Mann-Whitney	0,241	No
IL6 w/ **Position** at T0	Mann-Whitney	0,722	No
IL6+IL1β **Position** at T0	Mann-Whitney	0,337	No

Table 109 - Correlations between Interleukin Levels and anatomical position (Maxilla/Mandible at T2)

Interleukin (IL)	Test	*P*-Value	Correlation
IL-1β with **Position** atT2	Mann-Whitney	0,550	No
IL6 with **Position** at T2	Mann-Whitney	0,908	No
IL6+IL-1β **Position** at T2	Mann-Whitney	0,173	No

SECTION 5.9. SECONDARY OUTCOME MEASURES: ZIRCONIA, ACRYLIC AND TITANIUM INFLAMMATION LEVELS OF IL6 AND IL-1β AND CORRELATION TO MARGINAL BONE LOSS (MBL) AND THE DURATION OF SURGERY - HYPOTHESIS AND RESULTS

Section 5.9.1. Hypothesis

Correlation between Time of Surgery (duration of surgery), marginal bone loss (MBL1 and MBL2) and inflammation levels

With the Duration of surgery as the central variable and independent of the abutment material placed three groups were considered: **(9-15mn) Vs (15-20mn) Vs (+20mn)**)

Relating Duration of surgery **to Marginal Bone loss, independent of the material.**

Specific aim 1: To relate Duration of surgery **to Overall Marginal Bone Loss (MBL1, MBL2) at T2 (8 weeks)**

H0: There is no difference between Duration of surgery (in the two given groups) and marginal bone loss in implants placed under the standard protocol.

H1 There is a difference between Duration of surgery (in the three given groups) and marginal bone loss in implants placed under the standard protocol.

Relating Duration of surgery to Inflammatory Levels.

Specific aim 2: To relate Duration of surgery **to Overall Inflammatory Levels at T2 (8 weeks)**

H0: There is no difference between Duration of surgery (in the three given groups) and overall inflammatory levels (IL-1β+IL6) in implants placed under the standard protocol.

H1: There is a difference between Duration of surgery (in the two given groups) and overall inflammatory levels (IL-1β +IL6) in implants placed under the standard protocol.

Specific aim 3: To relate duration of surgery to Interleukin IL-1β Inflammatory Levels at T2 (8 weeks)

H0: There is no difference between Duration of surgery (in the three given groups) and inflammatory levels (IL-1β) in implants placed under the standard protocol.

H1: There is a difference between Duration of surgery (in the three given groups) and inflammatory levels (IL-1β) in implants placed under the standard protocol.

Specific aim 4: To relate duration of surgery with Interleukin IL6 Inflammatory Levels at T2 (8 weeks).

H0: There is no difference between Duration of surgery (in the three given groups) and inflammatory levels (IL6) in implants placed under the standard protocol.

H1: There is a difference between Duration of surgery (in the two given groups) and inflammatory levels (IL6) in implants placed under the standard protocol.

Specific aim 5: To relate duration of surgery to Overall Inflammatory Levels at T0 (Baseline).

H0: There is no difference between Duration of surgery (in the three given groups) and overall inflammatory levels (IL-1β+IL6) in implants placed under the standard protocol.

H1: There is a difference between Duration of surgery (in the two given groups) and overall inflammatory levels (IL-1β+IL6) in implants placed under the standard protocol.

Specific aim 6: To relate duration of surgery to Interleukin IL-1β Inflammatory Levels at T0 (Baseline)

H0: There is no difference between Duration of surgery (in the two given groups) and inflammatory levels (IL-1β) in implants placed under the standard protocol.

H1: There is a difference between Duration of surgery (in the three given groups) and inflammatory levels (IL-1β) in implants placed under the standard protocol.

Specific aim 7: To relate duration of surgery to Interleukin IL6 Inflammatory Levels at T0 (Baseline).

H0: There is no difference between Duration of surgery (in the three given groups) and inflammatory levels (IL6) in implants placed under the standard protocol.

H1: There is a difference between Duration of surgery (in the three given groups) and inflammatory levels (IL6) in implants placed under the standard protocol

Section 5.9.2. Results

In this section, we wanted to correlate inflammatory levels with the overall duration of surgery and marginal bone loss (MBL1 and MBL2). Table 110 shows the raw data collected divided by time and material used.

Table 110 - Collected data relating duration of surgery (in min) and the type of healing abutment placed.

Tooth	Material	Time
1	A	12
2	A	13
3	A	15

4	Z	12
5	T	30
6	T	13
7	T	16
8	A	12
9	A	30
10	T	11
11	A	12
12	A	25
13	Z	16
14	T	9
15	Z	15
16	Z	20
17	T	12
18	Z	18
19	Z	18
20	T	12
21	Z	11
22	A	18
23	T	12
24	Z	12
25	Z	16
26	T	13
27	A	25
28	T	13
29	Z	9
30	A	14
31	T	25
32	T	12
33	Z	15
34	Z	16
35	T	25
36	A	15
37	T	13
38	Z	13
39	A	9
40	Z	12
41	Z	11

42	Z	13
43	Z	13
44	A	13
45	A	12
46	Z	10
47	A	12
48	T	12
49	T	15
50	A	25
51	T	12
52	A	11
53	T	10
54	Z	23
55	Z	23
56	Z	14
57	A	10
58	A	11
59	Z	20
60	A	12

Correlation Between Duration of Surgery and marginal bone resorption

The duration of surgery was divided into 3 main groups from 9-15 minutes, from 15-20 minutes and over 20 minutes. There was an unequal sample size for the theseor time frames of 35 samples for 9-15 minutes, 14 samples for 15-20, with only nine implants taking more than 20 minutes to place.

With regard to marginal bone resorption, the results were very similar between time frames (9,27 mm, 10,14 mm and 11,35 mm table 111) resulting in no statistical differences between these time frames (table 112).

Table 111 - Mean average and standard deviation for Interleukins and time frame, and marginal bone loss related Surgery time

Duration of surgery	MBL1	MBL2	IL6 T0	IL6 T2	IL-1β T0	IL-1β T2	IL6+ IL-1β T0	IL6+ IL-1β T2
9-14	9,27 ±9,16	7,50 ±10,57	5,65 ±5,68	6,15 ±13,89	5,20 ±4,02	46,03 ±53,84	10,86 ±7,93	52,18 ±56,15
15-19	10,14 ±7,55	8,29 ±8,73	7,70 ±5,35	2,70 ±5,62	4,40 ±2,59	26,00 ±31,94	12,10 ±6,12	28,70 ±35,43
>20	11,35 ±9,73	7,00 ±10,56	6,67 ±4,61	8,75 ±13,81	6,33 ±4,79	41,50 ±52,43	13,00 ±8,44	50,25 ±59,90

Max- Maxilla Mand- Mandible MBL1 - Marginal Bone Loss (total in mm) MBL2- Marginal Bone Loss (only implant exposed in mm)

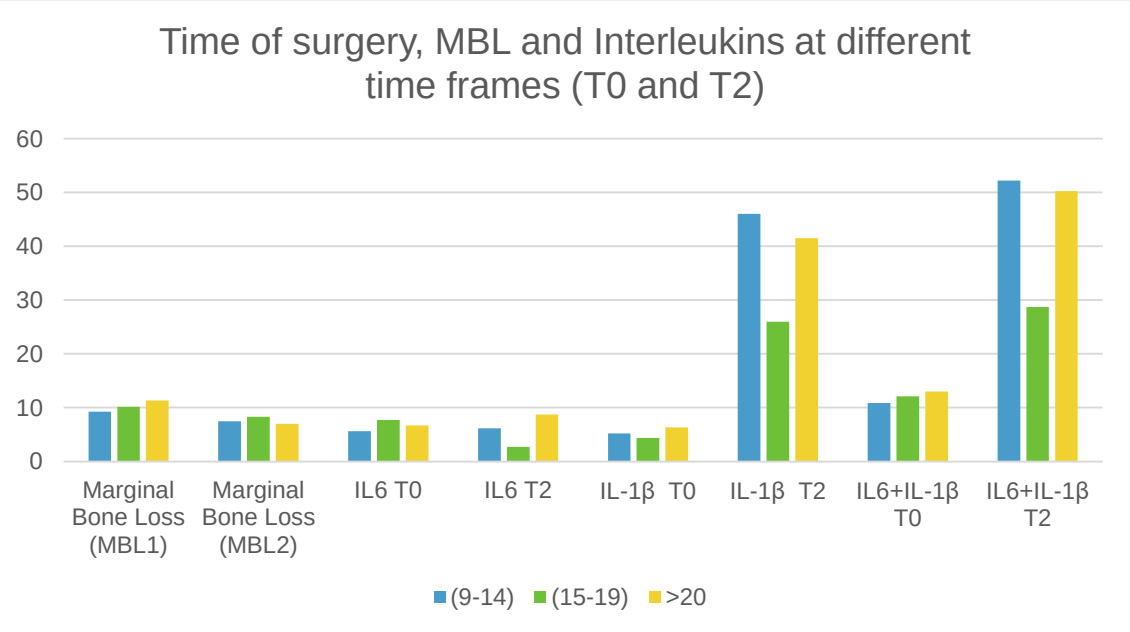


FIGURE 199 - Overall Interleukin variation at different time frames T0 and T2. Comparison with marginal bone loss (MBL1 and MBL2)

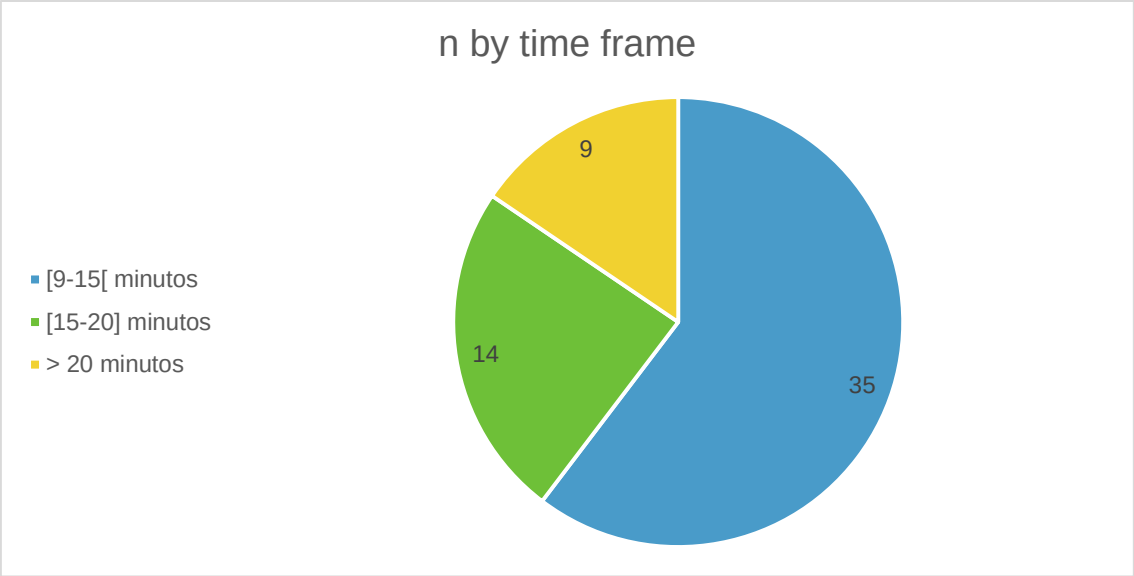


FIGURE 200 - Overall distribution of healing abutments by time.

Correlation Between Duration of Surgery and Inflammatory patterns

Duration does not significantly influence marginal bone loss and in no case, did the duration influence the indicated inflammatory variables.

All e results either at T0 and at T2 presented very similar IL concentrations either IL-1β, IL6 and total IL-1β +IL6. (table 113 and 114)

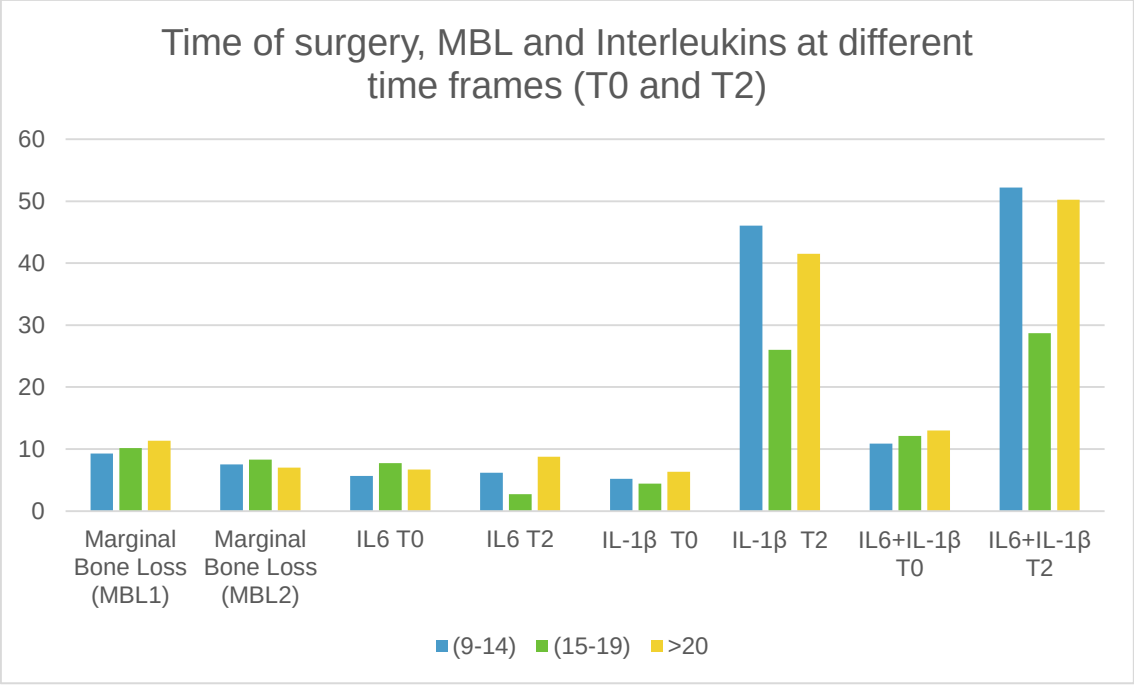
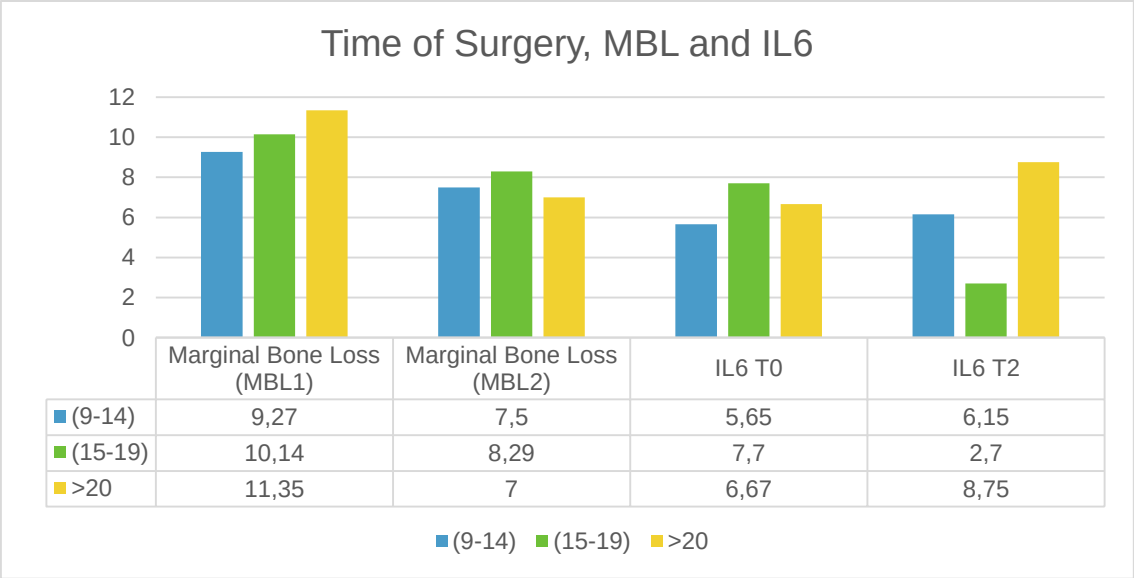
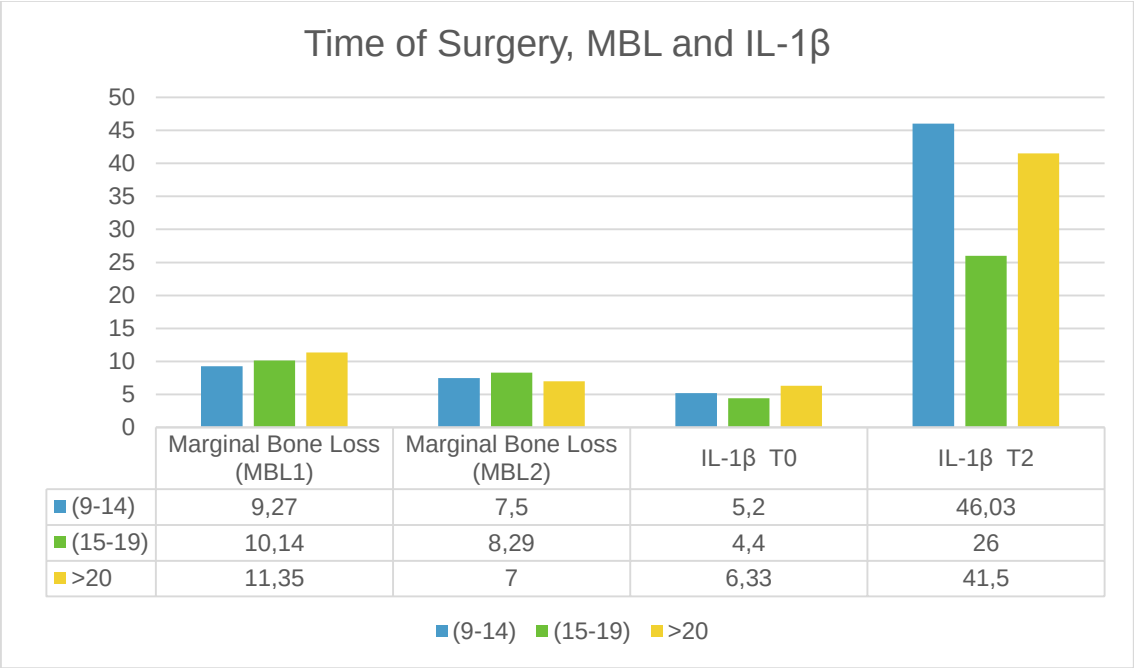


FIGURE 201 - Overall marginal bone loss and inflammatory variation at different time frames between the intervals of surgery duration.



	Marginal Bone Loss (MBL1)	Marginal Bone Loss (MBL2)	IL6 T0	IL6 T2
(9-14)	9,27	7,5	5,65	6,15
(15-19)	10,14	8,29	7,7	2,7
>20	11,35	7	6,67	8,75

FIGURE 202 - Overall marginal bone loss and inflammatory variation of IL6 between the intervals of surgery duration.



	Marginal Bone Loss (MBL1)	Marginal Bone Loss (MBL2)	IL-1β T0	IL-1β T2
(9-14)	9,27	7,5	5,2	46,03
(15-19)	10,14	8,29	4,4	26
>20	11,35	7	6,33	41,5

FIGURE 203 - Overall marginal bone loss and inflammatory variation of IL-1β between the intervals of duration of surgery.

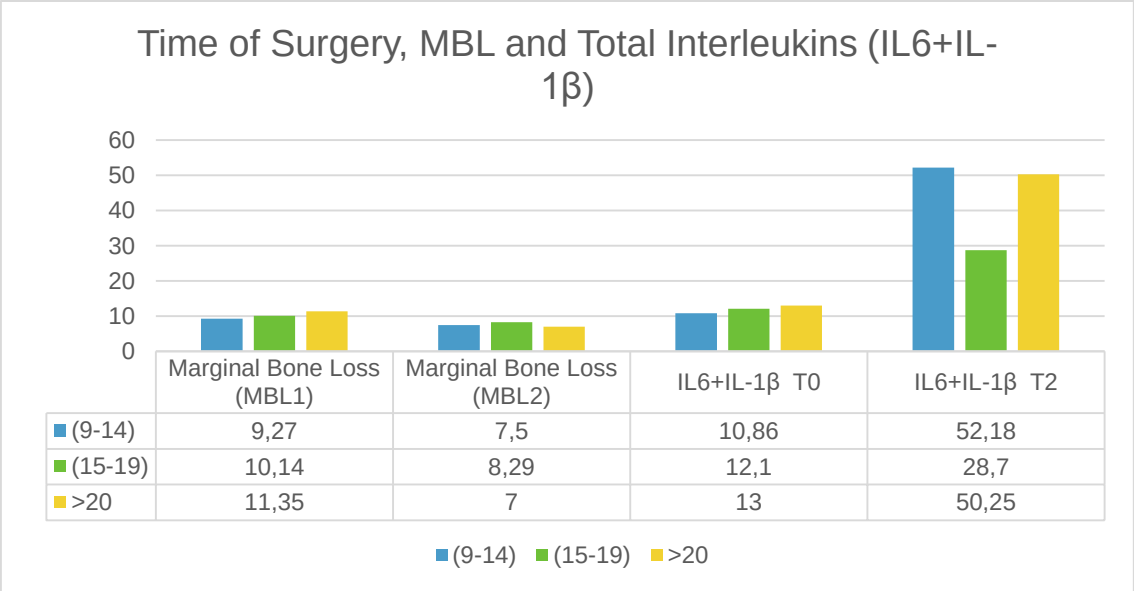


FIGURE 204 - Overall marginal bone loss and inflammatory variation of IL6 over the different intervals of duration of surgery.

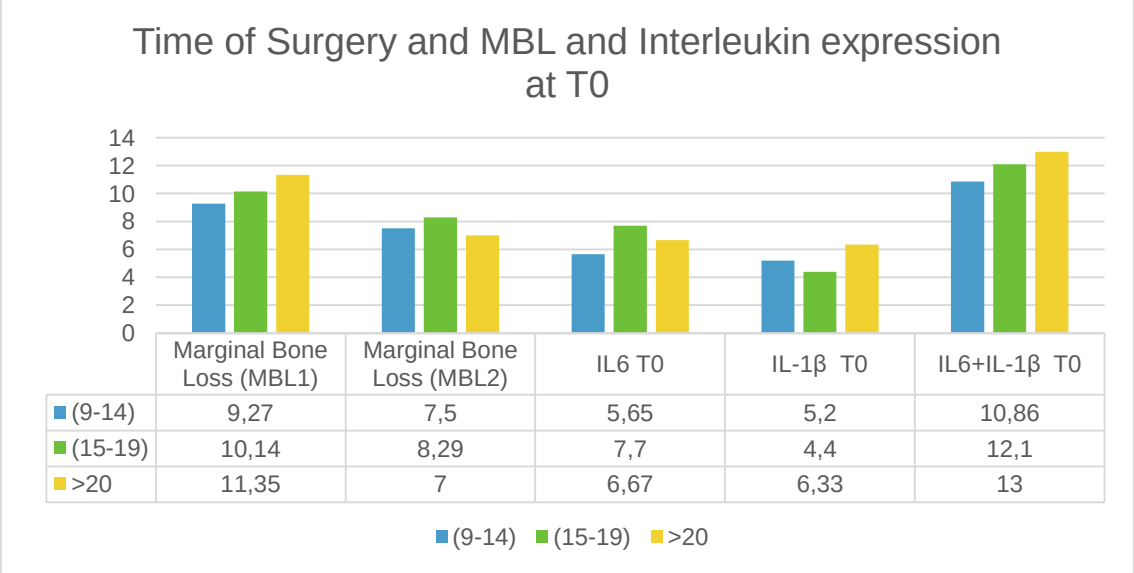
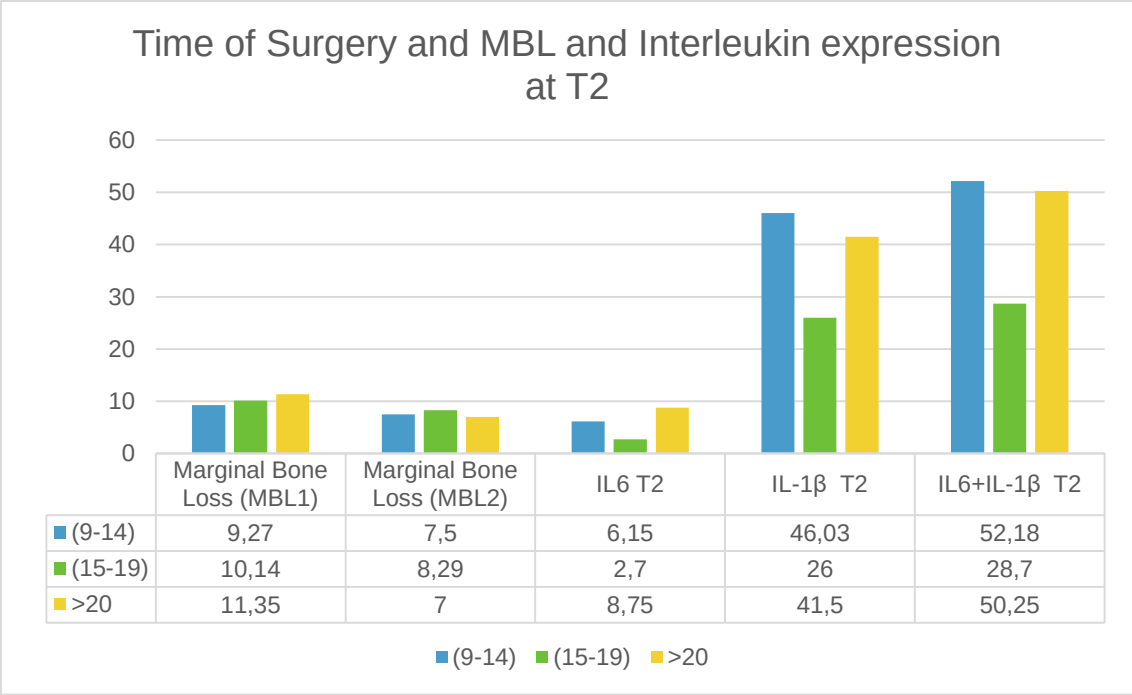


FIGURE 205 - Overall marginal bone loss and inflammatory variation of IL over the different intervals of duration of surger at T0 baseline.



	Marginal Bone Loss (MBL1)	Marginal Bone Loss (MBL2)	IL6 T2	IL-1β T2	IL6+IL-1β T2
(9-14)	9,27	7,5	6,15	46,03	52,18
(15-19)	10,14	8,29	2,7	26	28,7
>20	11,35	7	8,75	41,5	50,25

FIGURE 206 - Overall marginal bone loss and inflammatory variation of IL over the different intervals of duration of surgery at T2 (8 weeks**)**

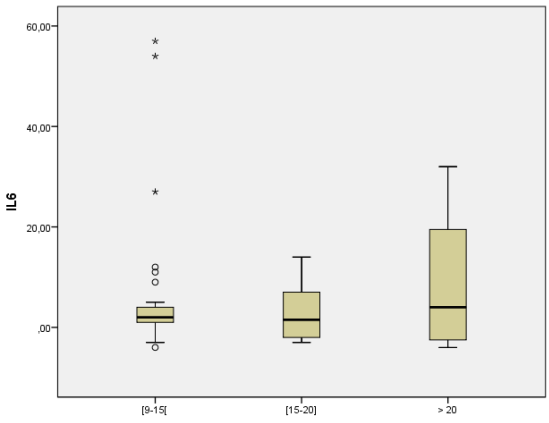


FIGURE 207 - Boxplot showing the interquartile differences of IL6 variation with duration of surgery

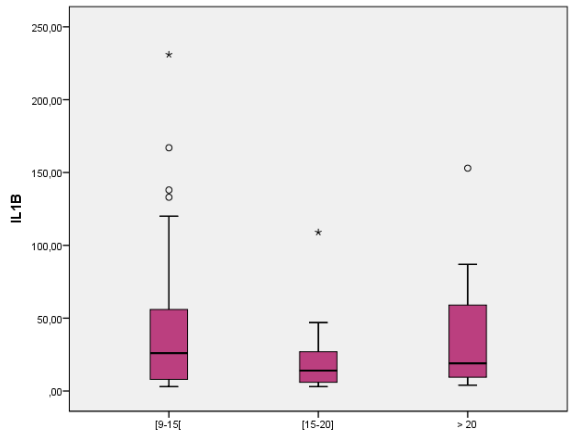


FIGURE 208 - Boxplot showing the interquartile differences of IL-1β variation with duration of surgery

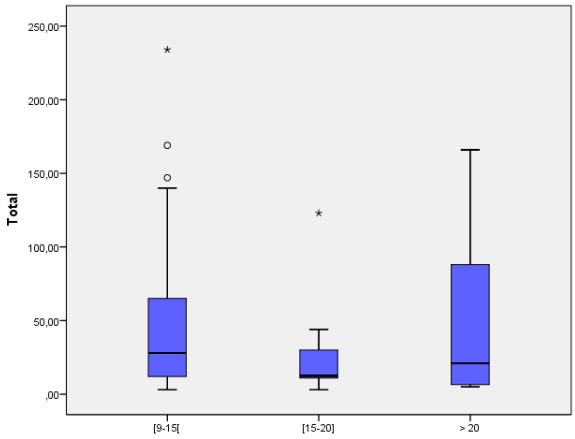


FIGURE 209 - Boxplot showing the interquartile differences of IL6+IL-1β variation with duration of surgery

Table 112 - Correlations between MBL (MBL1 and MBL2) and Duration of Surgery across all categories (9-14,12-19 and > 20)

MBL	Test	P-Value	Correlation
MBL1 with **Time**	kruskall-Wallis	0,693	No
MBL2 with **Time**	kruskall-Wallis	0,814	No

Table 113 - Correlations between Interleukin Levels and Duration of Surgery across all categories (9-14,12-19 and > 20) at T0

Interleukin (IL)	Test	P-Value	Correlation
IL-1β with **Time** at T0	kruskall-Wallis	0,764	No
IL6 with **Time** at T0	Anova	0,563	No
IL6+ IL-1β**Position** at T0	kruskall-Wallis	0,750	No

Table 114 - Correlations between Interleukin Levels and anatomical Duration of Surgery across all categories (9-14,12-19 and > 20) at T2

Interleukin (IL)	Test	P-Value	Correlation
IL-1β with **Time** at T2	kruskall-Wallis	0,489	No
IL6 with **Time** at T2	kruskall-Wallis	0,798	No
IL6+I IL-1β **Time** at T2	kruskall-Wallis	0,399	No

SECTION 5.10. SECONDARY OUTCOME MEASURES: ZIRCONIA, ACRYLIC AND TITANIUM INFLAMMATION LEVELS OF IL6 AND IL-1β AND CORRELATION TO MARGINAL BONE LOSS IMPLANT STABILITY (ISQ)- HYPOTHESIS AND RESULTS

Section 5.10.1. Hypothesis

Correlation between implant stability (measured in ISQ units), marginal bone loss (MBL) and inflammation levels.

Specific aim 1: With implant stability as the central variable and independent of the abutment material to find a correlation between Implant stability and marginal bone loss at Day 0 (baseline)

H0: There is no correlation between high insertion torque levels at day 0 (baseline) and increased marginal bone loss, in implants placed under the standard protocol.

H1: There is a correlation between high insertion torque levels at day 0 (baseline) and increased marginal bone loss, in implants placed under the standard protocol.

Specific aim 1: With implant stability as the central variable and independent of the abutment material to find a correlation between Implant stability and marginal bone loss at T2 (8 weeks)

H0: There is no correlation between high insertion torque levels at day 1 (T2-8weeks) and increased marginal bone loss, in implants placed under the standard protocol.

H1: There is a correlation between high insertion torque levels at day 1 (T2-8weeks) and increased marginal bone loss, in implants placed under the standard protocol.

Specific aim 2: With implant stability as the central variable and independent of the abutment material to find a correlation between Implant stability and

inflammatory levels **in Overall Inflammation parameters (IL6+IL-1β) at Day 0 (Baseline)**.

H0: There is no correlation between high insertion torque levels and increased inflammatory levels (IL6+IL-1β) at Day 0 (Baseline) in implants placed under the standard protocol.

H1: H0: There is no correlation between high insertion torque levels and increased inflammatory levels (IL6+IL-1β) at Day 0 (Baseline) in implants placed under the standard protocol.

Specific aim 3: With implant stability as the central variable and independent of the abutment material to find a corellation between Implant stability and inflammatory levels **in overall Inflammation parameters (IL6+IL-1β) At T2 (8 weeks)**

H0: There is no correlation between high insertion torque levels and increased inflammatory levels (IL6+IL-1β) at Day 1 (T2) in implants placed under the standard protocol.

H1: H0: There is no correlation between high insertion torque levels and increased inflammatory levels (IL6+IL-1β) at Day 1 (T2) in implants placed under the standard protocol.

Specific aim 4: To compare insertion torque levels to Inflammation parameters (IL6) at Day 0 (Baseline).

H0: There is no correlation between high insertion torque levels and increased inflammatory levels (IL6) at Day 0 (Baseline) in implants placed under the standard protocol.

H1: H0: There is no correlation between high insertion torque levels and increased inflammatory levels (IL6) at Day 0 (Baseline) in implants placed under the standard protocol.

Specific aim 5: To compare insertion torque levels to Inflammation parameters (IL6) at T2 (8 weeks).

H0: There is no correlation between high insertion torque levels and increased inflammatory levels (IL6) at Day 1 (T2) in implants placed under the standard protocol.

H1: H0: There is a correlation between high insertion torque levels and increased inflammatory levels (IL6) at Day 1 (T2) in implants placed under the standard protocol.

Specific aim 6: To compare insertion torque levels to Inflammation parameters (IL-1β) at Day 0 (Baseline).

H0: There is no correlation between high insertion torque levels and increased inflammatory levels (IL-1β) at Day 0 (Baseline) in implants placed under the standard protocol.

H1: H0: There is a correlation between high insertion torque levels and increased inflammatory levels (IL-1β) at Day 0 (Baseline) in implants placed under the standard protocol.

Specific aim 7: To compare insertion torque levels to Inflammation parameters (IL-1β) at T2 (8 weeks).

H0: There is no correlation between high insertion torque levels and increased inflammatory levels (IL-1β) at Day 1 (T2) in implants placed under the standard protocol.

H1: H0: There is a correlation between high insertion torque levels and increased inflammatory levels (IL-1β) at Day 1 (T2) in implants placed under the standard protocol.

Specific aim 8: With implant stability as the center variable and correlating with the abutment material. (Z, A and T)

H0: There is no difference between implant stability (measured in ISQ) when comparing titanium with zirconia and acrylic in implants placed under the standard protocol.

H1: There is a difference between implant stability (measured in ISQ) when comparing titanium with zirconia and acrylic in implants placed under the standard protocol

Correlate implant stability with other variables such as anatomical position, gender and age.

Specific aim 9: To compare Implant stability and Anatomical Position at baseline (T0)

H0: There is no difference between implant stability (measured in ISQ) when comparing implants placed in the maxilla and in the mandible, under the standard protocol.

H1: There is a difference between implant stability (measured in ISQ) when comparing implants placed in the maxilla and in the mandible, under the standard protocol.

Specific aim 10: At 8Weeks (T2)

H0: There is no difference between implant osseointegration values (measured in ISQ) when comparing implants placed in the maxilla and in the mandible, under the standard protocol.

H1: There is a difference between osseointegration values (measured in ISQ) when comparing implants placed in the maxilla and in the mandible, under the standard protocol.

Specific aim 11: Implant stability and Age At baseline (T0)

H0: There is no difference between implant stability (measured in ISQ) when comparing implants placed in patients under 65 compared to patients above or equal to 65-years old, under the standard protocol.

H1: There is a difference between implant stability (measured in ISQ) when comparing implants placed in patients under 65 compared to patients above or equal to 65-years-old, under the standard protocol.

Specific aim 11: Implant stability and Age At 8 Weeks (T2)

H0: There is no difference between implant stability (measured in ISQ) when comparing implants placed in patients under 65 compared to patients above or equal to 65-years-old, under the standard protocol.

H1: There is a difference between implant stability (measured in ISQ) when comparing implants placed in patients under 65 compared to patients above or equal to 65-years-old, under the standard protocol.

Specific aim 12: Implant stability and Age Implant stability and Gender At baseline (T0)

H0: There is no difference between implant stability (measured in ISQ) when comparing implants placed in male patients compared to female patients under the standard protocol.

H1: There is a difference between implant stability (measured in ISQ) when comparing implants placed in male patients compared to female patients, under the standard protocol.

Specific aim 13: Implant stability and Age Implant stability and Gender At 8Weeks (T2)

H0: There is no difference between implant stability (measured in ISQ) when comparing implants placed in male patients compared to female patients, under the standard protocol.

H1: There is a difference between implant stability (measured in ISQ) when comparing implants placed in male patients to female patients, under the standard protocol.

Section 5.10.2. Results

For comparing primary and secondary stability in each abutment/implant complex, three measurements in each time frame were taken at T0 and T2. The mean average of these was calculated and the value used to find the gain on each abutment as displayed in table 115.

Table 115 - Collected data regarding Primary stability (PSB) at T0 and Secondary stability (SS) at T2 (8weeks)

Implant #	Tooth #	Material	ISQ Before			Ave	ISQ After			Ave	Gain
1	47	A	63	67	63	64,33	68	68	64	66,67	2,33
2	45	A	74	59	59	64	62	62	62	62	-2
3	25	A	62	62	62	62	76	76	73	75	13
4	16	Z	53	45	52	50	65	58	58	60,33	10,33
5	36	T	63	63	63	63	66	74	66	68,67	5,67
6	25	T	79	79	79	79	81	80	81	80,67	1,67
7	37	T	63	58	63	61,33	70	70	70	70	8,67
8	37	A	60	60	60	60	75	71	71	72,33	12,33
9	46	A	76	66	75	72,33	62	62	63	62,33	-10
10	17	T	43	43	43	43	68	68	70	68,67	25,67
11	24	A	62	70	62	64,67	70	70	70	70	5,33
12	22	A	59	59	65	61	80	80	80	80	19
13	36	Z	63	64	63	63,33	70	70	64	68	4,67
14	26	T	53	58	70	60,33	68	68	68	68	7,67
15	46	Z	70	75	70	71,67	70	70	70	70	-1,67
16	11	Z	54	54	54	54	70	64	68	67,33	13,33
17	25	T	48	46	48	47,33	65	65	65	65	17,67
18	36	Z	83	83	83	83	71	71	71	71	-12
19	15	Z	72	73	74	73	68	70	70	69,33	-3,67
20	37	T	77	74	77	76	60	60	68	62,67	-13,33
21	46	Z	77	76	77	76,67	65	65	62	64	-12,67
22	16	A	48	48	48	48	67	67	67	67	19
23	26	T	39	41	39	39,67	58	59	55	57,33	17,67
24	14	Z	77	76	77	76,67	58	57	58	57,67	-19
25	14	Z	61	67	61	63	63	54	62	59,67	-3,33

26	24	T	75	76	76	75,67	52	58	62	57,33	-18,33
27	25	A	62	62	62	62	68	69	65	67,33	5,33
28	15	T	83	83	83	83	65	65	65	65	-18
29	36	Z	64	64	42	56,67	72	72	65	69,67	13
30	36	A	75	75	75	75	83	83	83	83	8
31	24	T	61	61	61	61	75	74	71	73,33	12,33
32	46	T	73	70	71	71,33	69	69	67	68,33	-3
33	46	Z	70	70	70	70	70	70	70	70	0
34	16	Z	64	64	67	65	61	60	63	61,33	-3,67
35	24	T	61	61	62	61,33	59	59	59	59	-2,33
36	45	A	65	66	60	63,67	70	70	72	70,67	7
37	26	T	63	63	67	64,33	67	67	68	67,33	3
38	24	Z	63	63	63	63	76	76	80	77,33	14,33
39	16	A	52	52	61	55	70	70	71	70,33	15,33
40	25	Z	63	49	35	49	72	72	58	67,33	18,33
41	15	Z	56	53	53	54	56	55	56	55,67	1,67
42	23	Z	46	46	48	46,67	69	69	69	69	22,33
43	14	Z	70	70	70	70	71	72	72	71,67	1,67
44	14	A	70	73	90	77,67	74	74	75	74,33	-3,33
45	36	A	72	72	71	71,67	68	68	67	67,67	-4
46	25	Z	60	60	65	61,67	67	67	67	67	5,33
47	24	A	56	59	56	57	62	58	57	59	2
48	35	T	61	61	64	62	0	0	0	0	-62
49	45	T	57	57	58	57,33	78	80	80	79,33	22
50	15	A	67	69	67	67,67	73	73	73	73	5,33
51	44	T	48	62	62	57,33	67	67	65	66,33	9
52	24	A	65	60	65	63,33	64	65	68	65,67	2,33
53	25	T	42	42	42	42	70	70	70	70	28
54	26	Z	53	53	60	55,33	59	60	58	59	3,67
55	25	Z	70	73	70	71	65	72	65	67,33	-3,67
56	46	Z	59	59	58	58,67	0	0	0	0	-58,67
57	15	A	68	60	59	62,33	71	72	72	71,67	9,33
58	35	A	61	70	62	64,33	71	71	70	70,67	6,33
59	12	Z	48	54	54	52	56	61	56	57,67	5,67
60	34	A	63	63	63	63	73	73	73	73	10

The mean average and SD of each abutment is shown in table 117 where we can see that the average is similar in the three biomaterials.

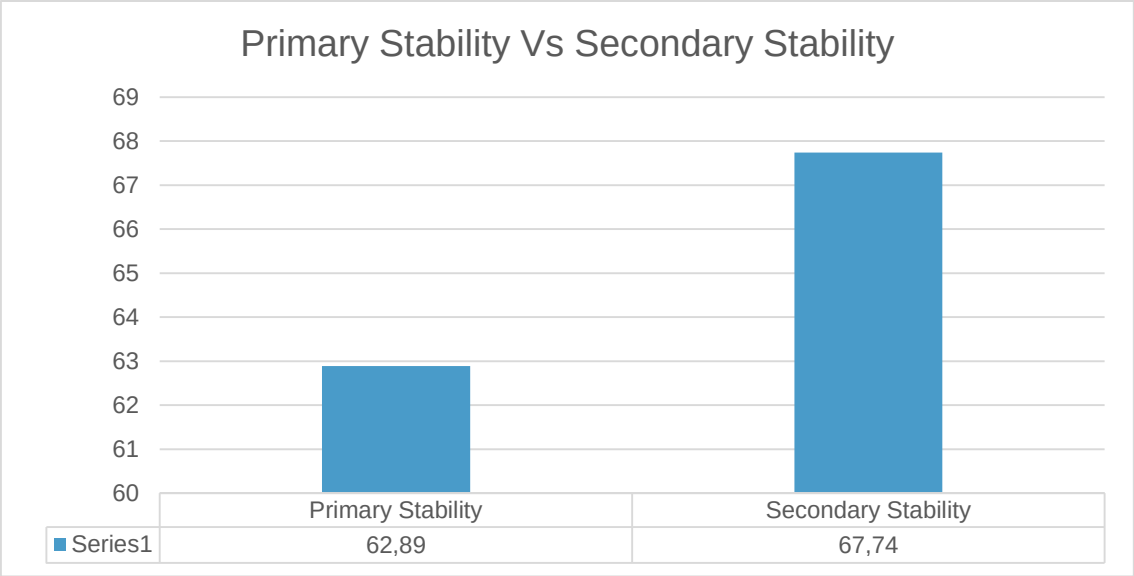


FIGURE 210 - Overall comparison between primary and secondary stability independent of the material used.

Compare Primary stability from T0 to secondary stability T2 across all groups

When we compared all implants independent of implant abutment we saw an increase from T0 to T2 (62 to 67 units ISQ) that were statistically significantly different.

The final *p*-value = 0.001 showed that there were significant differences in stability between T0 and T2, and, on average, stability was significantly higher at T2 than at T0.

Table 116 - Correlations between Primary stability T0 and secondary stability T2

	Test	
Stability before T0/after T2	Paired T-test	0,001
*across all groups		

Compare Primary stability from T0 to secondary stability T2 divided by biomaterial

The results by biomaterial are displayed in table 117 and we can see that the values are very similar and the difference between T0 and T2 were in the same range.

Although Acrylic showed a tendency to have more primary and secondary stability the biomaterial did not influence stability in any way (primary or secondary stability).

Table 117 shows the mean average for primary and secondary stability by biomaterial and the related MBL and inflammation values.

Table 117 - Collected data relating primary stability and different abutment materials

	Primary Stability (T0)	Secondary Stability (T2)
T	61,35±13,04	67,47±6,52
A	63,95±6,78	70,08±5,78
Z	63,12±10,31	65,73±5,71

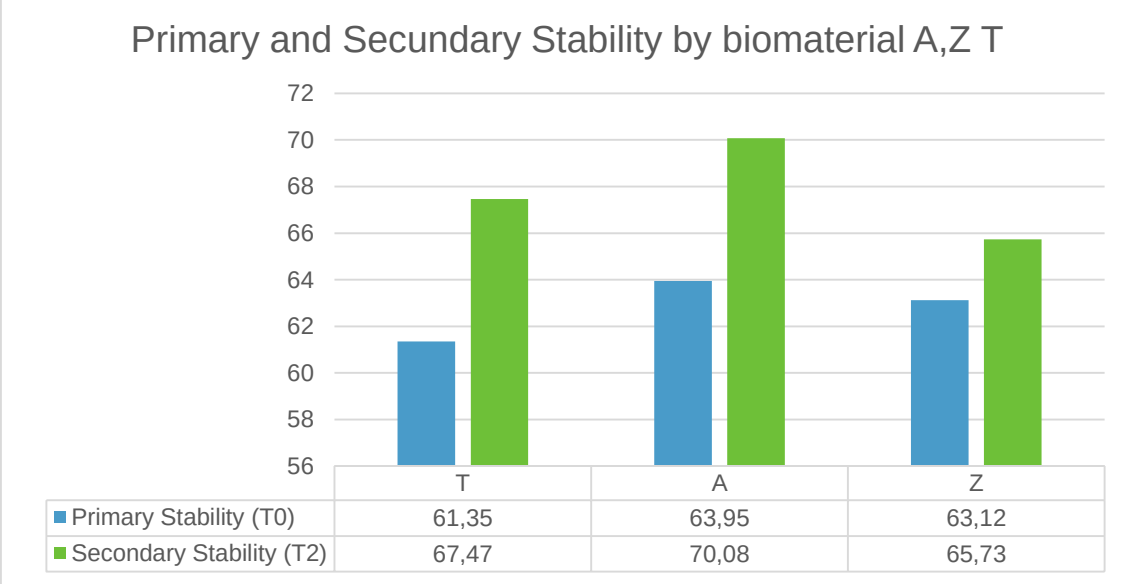


FIGURE 211 - Primary and secondary stability in each healing abutment.

Compare Primary stability from T0 to secondary stability T2 and marginal bone resorption

The first parameter was to evaluate PSB and SS with MBL 1 and MBL2 (table 118 and fig. 192).

The results showed that at T0 and T2, stability was not significantly related to marginal bone loss (table 121).

The second parameter was to see if there were any alterations in the inflammation pattern and correlate that with PSB and SS.

Table 118 - Collected data relating primary stability, marginal bone loss and inflammation in different abutment material at T0

	Primary Stability (T0)	Secondary Stability (T2)	MBL1	MBL2	IL6 pg/ml T0	IL-1β pg/ml T0	IL6+IL1β pg/ml T0
T	61,35 ±13,04	67,47 ±6,52	10,48 ± 12,05	8,79 ± 13,13	4,65 ± 4,57	6,35 ± 5,37	11 ± 8,59
A	63,95 ±6,78	70,08 ±5,78	10,40 ± 6,92	8,67 ± 9,04	7,63 ± 6,58	5,31 ± 3,16	12,95 ±7,78
Z	63,12 ±10,31	65,73 ±5,71	8,69 ± 7,43	5,65 ± 7,91	6,12 ± 4,64	4,11 ± 2,7	10,28 ± 6,6

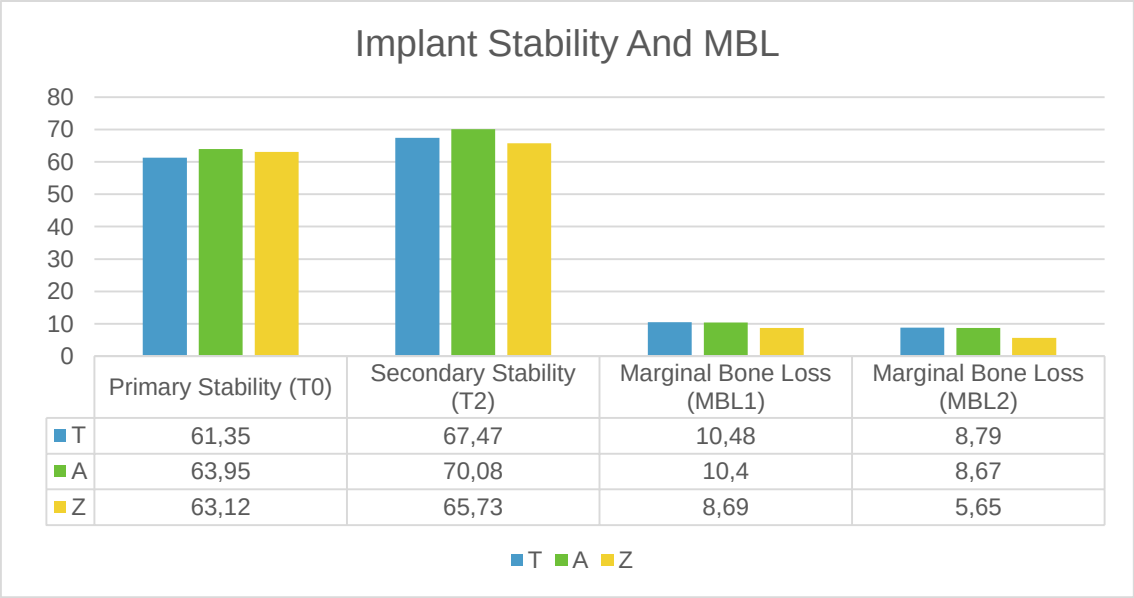


FIGURE 212 - Relation between marginal bone loss and primary and secondary stability

Compare Primary stability from T0 to secondary stability T2 and Inflammatory patterns.

One of the first conclusions was that at T0 (baseline), implant stability was not significantly related to marginal bone loss (either MBL1 or MBL2), nor was it related to inflammation, namely to IL6, IL-1β and in total. (table 119,120 and 122).

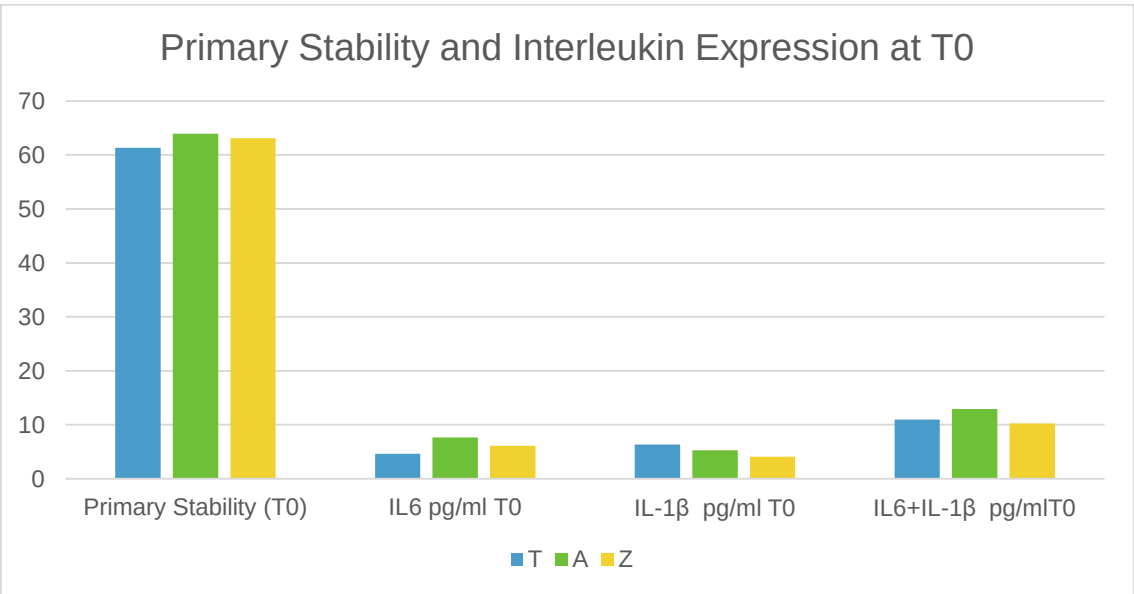


FIGURE 213 - Overall relation between primary stability and inflammation (interleukin expression) at T0

Table 119 - Correlations between Interleukin IL-1β,6 and total and Primary Stability

	Test	IL-1β	IL6	Total (IL-1β+IL6)
Stability	Pearson Correlation	0,134	0,586	0,248

Table 120 - Correlations between Interleukin IL-1β,6 and total and Secondary Stability

	Test	IL-1β	IL6	Total (IL-1β +IL6)
Stability	Pearson Correlation	0,623	0,248	0,965

Table 121 - Correlations between MBL and Primary Stability

	Test	MBL1	MBL2
Stability	Pearson Correlation	0,473	0,274

Table 122 - Correlations between stability and Biomaterial used at T0

	Test	Acrylic	Titanium	Zirconia
Stability	Anova		0,695*	

*across all groups

In addition, for secondary stability there were no differences between biomaterials as shown in table 123.

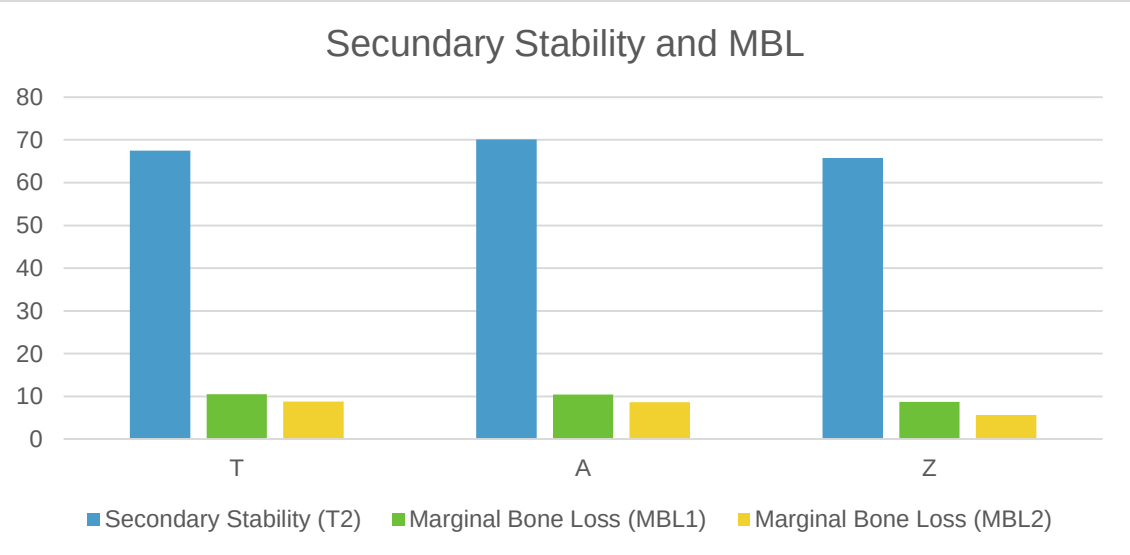


FIGURE 214 - Overall relation between secondary stability and marginal bone loss at T2

Table 123 - Collected data relating primary stability, marginal bone loss and inflammation in different abutment material at T2

	Primary Stability (T0)	Secondary Stability (T2)	MBL1	MBL2	IL6 pg/ml T2	IL-1β pg/ml T2	IL6+IL-1β pg/ml T2
T	61,35	67,47	10,48	8,79	4,06	64,75	68,81
	±13,04	±6,52	± 12,05	± 13,13	±7,99	±55,24	± 59,81
A	63,95	70,08	10,40	8,67	8,56	31,44	40
	±6,78	±5,78	± 6,92	± 9,04	±14,82	±33,40	± 39,66
Z	63,12	65,73	8,69	5,65	4,76	29,94	34,70
	±10,31	±5,71	± 7,43	± 7,91	±13,83	±54,07	± 55,99

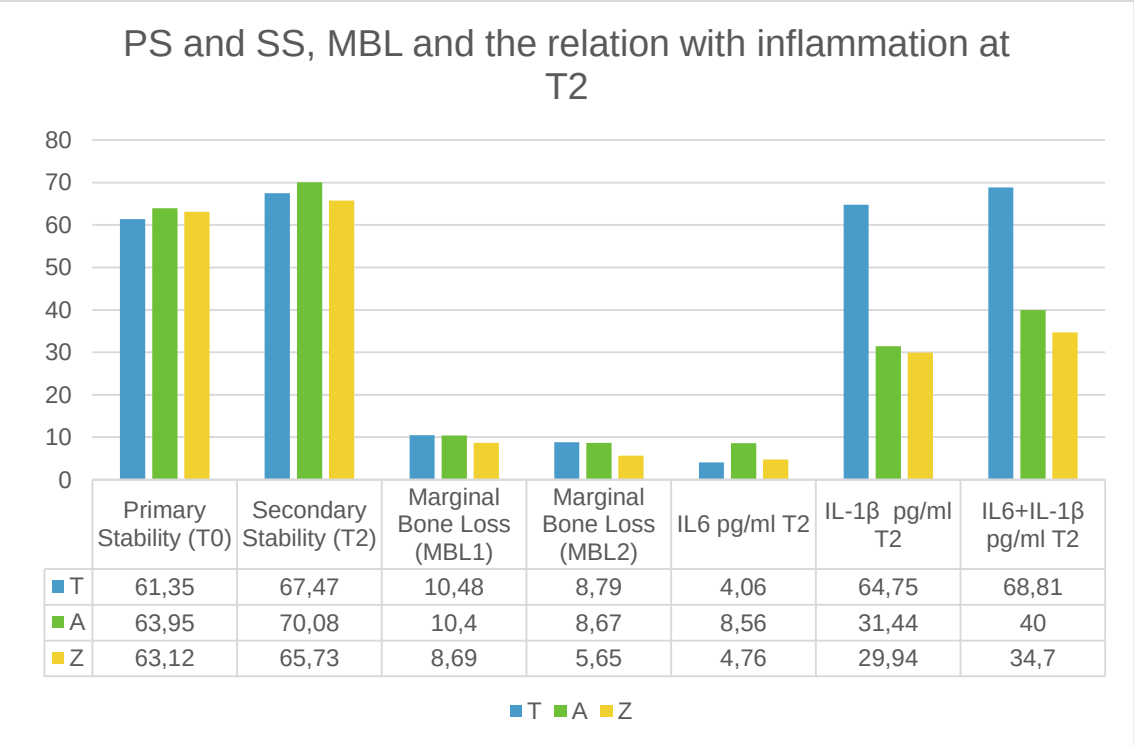


FIGURE 215 - Correlation between BL, inflammation and PSB/SS at T2 (8weeks)

Table 124 - Correlations between MBL and Secondary Stability

	Test	MBL1	MBL2
Stability	Pearson Correlation	0,215	0,245

Table 125 - Correlations between stability and Biomaterial used at T2

	Test	Acrylic	Titanium	Zirconia
Stability	Anova		0,075*	

*across all groups

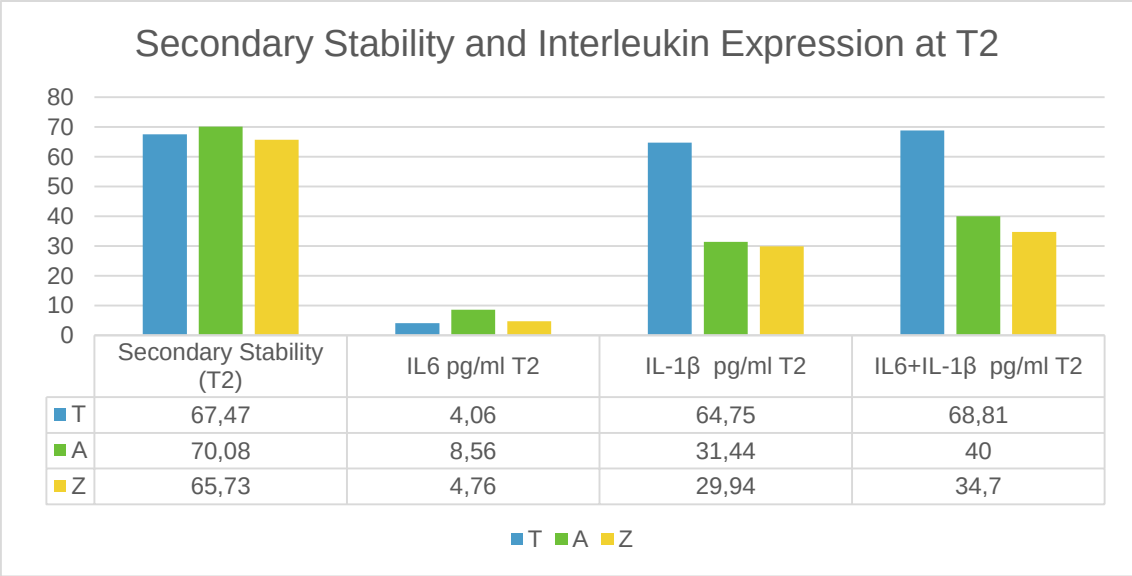


FIGURE 216 - Overall view of Interleukin variation and secondary (osseointegration) stability.

Table 126 - Correlations between Interleukin IL-1β,6 and total and Biomaterial used (Z, A, T) independent of the moment (primary stability T0 +secondary stability T2)

	Test	Acrylic	Titanium	Zirconia
Stability	Anova		0,311*	
*across all groups				

Compare Primary Stability and Secondary Stability with the position of the implant (mandible Vs Maxilla)

Table 127 - Collected data relating primary stability and Implant Position

	Primary Stability (T0)	Secondary Stability (T2)
Maxilla	60,55±11,10	66,56±6,62
Mandible	66,44±7,10	69,53±4,98

Compare Primary stability from T0 to secondary stability T2 and anatomical positions

When comparing the anatomical position, we found that stability differs with position at T0 being, on average, statistically significantly higher in the mandible than in the maxilla (table 128).

At T2 there were no statistical differences between them (table 129).

Table 128 - Correlations between primary stability T0 and implant position (Maxilla Vs Mandible)

	Test	Maxilla	Mandible
Stability	Mann-Whitney		0,039
*across all groups			

Table 129 - Correlations between Secondary stability T2 and implant position (Maxilla Vs Mandible)

	Test	Maxilla	Mandible
Stability	Mann-Whitney		0,072
*across all groups			

Compare Primary Stability and Secondary Stability with Age (65 Vs plus 65)

When these items are compared, stability values are very similar in both groups (under and beyond 65) with no statistical differences between them (fig. 197).

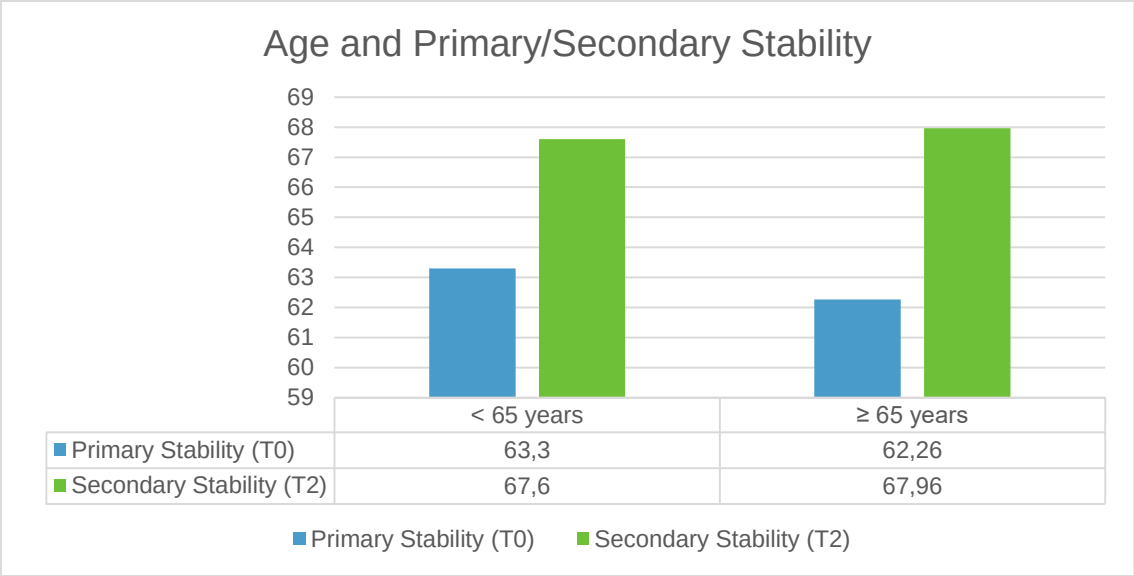


FIGURE 217 - The graphic shows a tendency for older people to experience more inflammation.

Table 130 - Correlations between primary stability and Age at T0

	Primary Stability (T0)	Secondary Stability (T2)
< 65 years	63,30±9,87	67,60±6,08
≥ 65 years	62,26±10,59	67,96±6,41

.

Table 131 - Correlations between primary stability and Age at T0

	Test	Age
Stability	T-test	0,703
*across all groups		

Table 132 - Correlations between Secondary stability and Age at T2

	Test	Age
Stability	T-test	0,831
*across all groups		

Compare Primary Stability and Secondary Stability with Gender (M Vs F)

When these items are compared, the stability values are very similar in both groups (male and female) with no statistical differences between them.(fig. 198)

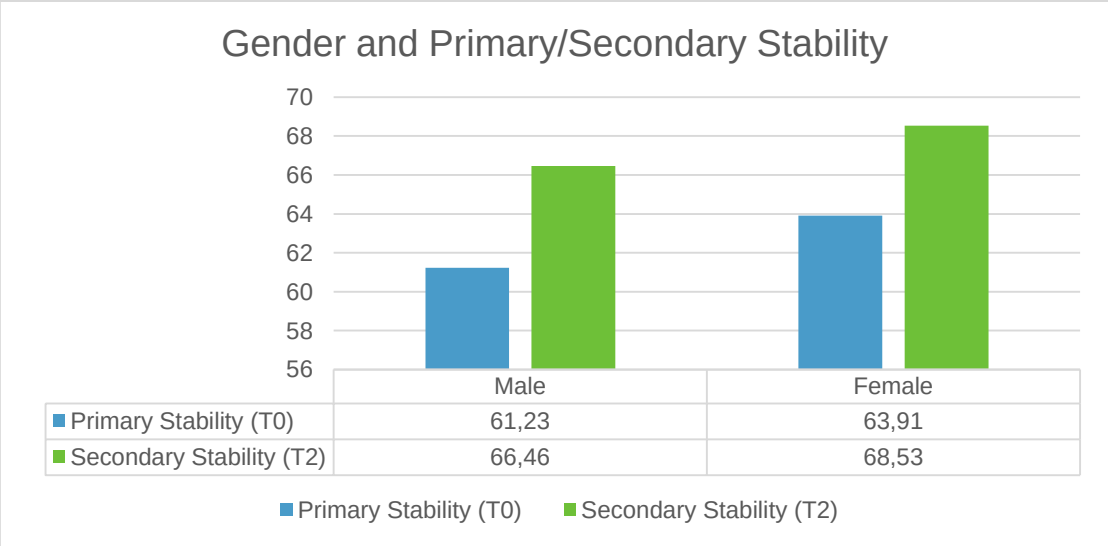


FIGURE 218 - Gender and primary and secondary stability

Table 133 - Correlations between primary stability and Age at T0

	Primary Stability (T0)	Secondary Stability (T2)
Male	61,23±10,28	66,46±4,93
Female	63,91±9,96	68,53±6,75

At T2, stability is not influenced by gender.

Table 134 - Correlations between primary stability and gender (Male/female)

	Test	Gender
Stability	Mann-Whitney	0,330
*across all groups		

Table 135 - Correlations between secondary stability and gender (Male/female)

	Test	Gender
Stability	Mann-Whitney	0,217
*across all groups		

CHAPTER 6. DISCUSSION

6.1. SECTION 5.1, 5.3, 5.4 DISCUSSION OF THE IMPACT OF DIFFERENT BIOMATERIALS ON THE OVERALL INFLAMMATORY RESPONSE TO DENTAL IMPLANTS (PCF, PICF AND BF MEASURE)

The results of our thesis in terms of inflammation levels are displayed in table 136 (Chen Phd) with the findings on IL-1β and IL6 expression in PICF, confronted with the literature up to 1 October 2017.

Table 136 - Comparison of peri-implant Interleukin concentrations in the literature

Study	N	IL6 (pg/ml)				IL-1β (pg/ml)			
		T0	T1 (2 weeks)	T2 (8weeks)	6month	T0	T1 (2weeks)	T2 (8weeks)	6month
Z(Chen Phd) +	18	6,17±4,64	-	4,76±13,83		4,11±2,7	-	29,94±54,07	
A(chen PhD) +	19	7,63±6,58	-	8,56±14,82		5,31±3,16	-	31,44±33,40	
T(Chen Phd)+	17	4,65±4,57	-	4,06 ± 7,99		6,35±5,37	-	64,75±55,24	
Overall (Chen Phd	54	6,20±5,43		6,01±12,58		5,24±3,91		55,41±49,85	
Emecen-Huja 2013*^	57	-	9±3	0,5±0,1		-	8±1	14±2	
Barwacz 2016 Zirconia	28				4,94±3,73				14,03±14,68
Barwacz 2016 Titanium	18				8,31±8,55				46.19±83,03
Panagakos 1996	50								59,47±15,55
Casado*** 2013	10								67,51±62,9
Melo 2011	31				0.32±0.59				2.04 ± 2.74
Kuppusamy 2015 (final ab)									57
Kuppusamy 2015 (healing ab)									97
Bielemann 2017****?	60		102,6 (0-573)	40,7 (0-784)			21,7 8 8(3,6-5,2)	13,5 (0,97)	
Ata-ali 2015					0,53±0,63				21,2±24,2

+refers to Biomet-Zimmer T3 implant internal hex

*Screw type, root form implants (Astra Tech, Moindal, Sweden; Straumann USA, LLC, Andover, MA, USA; Zimmer Dental, Carsbal, CA, USA

**Astra Tech Osseospeed, Astra Tech AB, Mölndal, Sweden

***hexternal hexagon; Titanium Fix Dental Implants; São José dos Campos, São Paulo, Brazil

**** ø2.9–10 mm Facility- NeoPorossurface, Neodent Osseointegrated Implants, Curitiba, Brazil

^ng/ml ? pg/ul

When the presence of interleukins was compared in the peri-implant crevicular fluid (PICF), two completely opposite situations were found with respect to the behavior of IL-1β and IL6.

In relation to IL6 concentrations on the day of surgery (baseline (T0)), the results showed higher concentration values for IL6 than for IL-1β at the same time point, which are in line with results obtained by other authors.

It has been extensively documented in the literature that IL6 occurs in response to acute trauma and disappears almost completely after 8 weeks. (Emecen-Huja et al. 2013)

This IL6 pattern of expression is the same, independent of biomaterial used in healing abutments Z, A, T.

In IL-1β, only a residual form of expression was observed at T0, but at T2 the behavior was completely the opposite when compared to IL6. On the day of implant placement IL-1β appeared in minimal concentrations, but increased in value, as a low-chronic infection established itself after 8 weeks.

This pattern of IL expression is not modified by the abutment biomaterial.

Although the overall pattern of IL-1β variation from T0 to T2 seems to be the same, the total final concentration values of IL expression at T2 alone, changed with the type of abutment placed.

The stronger performance (less inflammation) of zirconia in relation to acrylic and titanium can be explained by the good clinical integration that the zirconia ceramic materials are known to achieve.

The statistically significant difference between values (inflammatory levels measured in IL6 and IL-1β) expressed at T2 between titanium and zirconia demonstrates that zirconia is a more inert (less inflammation) material than titanium with respect to the inflammatory response.

Blanco et al. in 2016 (Blanco et al. 2016b) put forward a study to compare the histomorphometry of a zirconia versus a titanium abutment placed over a titanium dental implant, in a study design very similar to our own.

In the Blanco et al. in 2016 (Blanco et al. 2016b) study, no statistically significant differences in the heights of biological width were found, between the two biomaterials. That study “only” provided sectional data at a particular time frame, with no exact information on how the tissues communicated between each other in terms of cell biology and biochemistry.

However, a very important result from the Blanco et al. in 2016 (Blanco et al. 2016b) study is that the percentage of blood vessels was higher for T, in comparison to Z (5,11% ± 1,70 and 2,23% ± 0,98, respectively).

The biological plausibility is evident when we do a cross study between Blanco and our thesis. Blanco et al. in 2016 (Blanco et al. 2016b) found more vessels on the titanium and we found more inflammation (expressed in IL) which may make sense if we take into account the fact that (in our study) titanium causes a more pronounced immune response. Having this in mind is easy to understand that merging studies, Blanco et al. in 2016 (Blanco et al. 2016b) concluded more vessels, more blood and we concluded more IL6 and IL-1β in the titanium healing abutment/implant area.

In our study a primary objective was to demonstrate how the tissues signal between each other in terms of interleukins variation.

As Blanco et al. in 2016 (Blanco et al. 2016b) demonstrates, although they have the same histological behavior, the way they condition a host immune response is totally different.

Thus, with respect to the inflammatory expression of IL-1β, titanium biomaterials express a higher amount than the zirconia abutments, with acrylic in an intermediate position.

Results reporting the behavior of IL6 from T0 to T2 can be found in other publications, where the mean concentration drops from 9 pg/ml to 0.5 pg/ml (Emecen-Huja et al. 2013) and in a more recent publication from 102 pg/ml to 40.7 pg/ml (Bielemann et al. 2017), showing the same pattern as our study, although in our study the drop was not so pronounced.

When we compare the overall difference from T0 to T2 there is a drop of - 0,18 ± 12,9 pg/ml, leading us to conclude that IL6 expression is similar in both time frames. If we break it down by material we see that titanium changes from 4,65

± 4,57 pg/ml to 4,06 ± 7,99 pg/ml, Acrylic from 7,63±6,58 pg/ml to 8,56±14,82 pg/ml and zirconia from 6,17 ± 4,64 pg/ml to 4,76 ± 13,83 pg/ml.

Again, there is a tendency for a more pronounced decrease in inflammation with the zirconia abutment, although there was no statistical significance.

In our experience IL6 concentration and variation remained the same after 8 weeks (or at least it was very similar). One explanation for this is that the release of this interleukin into the medium is facilitated by the rapid appearance of acute inflammatory cells, such as macrophages and neutrophils.

Once established and modulated, the immune response (as in the case of an 8-week implant healing) is mediated by the late immune response with the appearance of B-lymphocytes among others, favoring the appearance of other signaling molecules such as IL-1β.

In our study, the behavior of IL6 between T0 and T2, does not change in terms of concentration levels, beginning with low concentrations and continuing low after eight weeks. The absence of statistically significant differences in the overall expression of inflammatory values of the implants, is also reflected when we break it by A, Z or T material.

Using a titanium, zirconia or acrylic healing abutment (all made in CAD-CAM) seems to produce no differences in the expression of IL6 to the crevicular medium from T0 toT2.

The results obtained from the IL6 are in agreement with the literature.

For example, Barwacz (Barwacz et al. 2015) obtained a value of 4,94 pg/ml after osseointegration, and also, 8,31 pg/ml when he changed from zirconia to titanium. In our case, there was no significant change in IL6 expression when titanium and zirconia were evaluated and values of 4,76 pg/ml and 4,06 pg/ml were obtained respectively, a similar situation to Barwacz.

Melo 2012 (Melo, Lopes, Shibli, Marcantonio Junior, et al. 2012) and Huja (Emecen-Huja et al. 2013) yielded residual values of IL6 of 0.32 pg/ml and 0.5 pg/ml after osseointegration, representing lower values than our study.

The differences found in concentrations of interleukins with respect to different times of analysis, as well as, after completion of osseointegration, can be explained, in part, by differences in work protocols.

One of the most important variation one, is the lack of a uniform methodology used in other articles, that do not distinguish brand, implant geometries and compare different situations (in the same study) that can produce different results (Emecen-Huja et al. 2013). Another parameter of evaluation, the interleukin extraction protocol, also varies from author to author.

For example, in our study, samples were collected and the method optimized through our animal sheep study, while several authors do not have an optimization protocol for collecting data, transportation or extraction methodology, which can be a source of interference, since we are talking about very low concentrations of interleukins in which any small variation can produce different results.

Despite some discrepancies in values, what has been proven (in the majority of the literature available) is that in peri-implant situations, the presence of IL6 is residual, although it can be found in the acute phase (baseline in the case of our study), it does not seem to appear in the mediation of chronic inflammatory phenomena such as the interaction between soft tissue and implant at T2.

There is a totally different scenario for the behavior of IL-1β.

When it comes to the IL-1β situation, and considering our results, it is easy to understand, that IL-1β is not an acute onset interleukin but a late onset one in a situation of chronic autoimmune response.

Being an interleukin mediated by chronic lineage molecules such as, lymphocytes, this molecule has a very distinct pattern from IL6.

It has been shown in our results that the biomaterial that is placed on the platform of the implant makes an inflammatory and statistically significant difference between the concentration of IL-1β at T0 and its concentration at T2.

The presence of this type of interleukin in such large quantities in the peri-implant sulcus at T2, reveals that the implant/abutment complex is directly

responsible for the presence of an inflammatory exudate with osteoclast properties, since this interleukin is a potent osteoclast activator.

The difference in the values of this interleukin in blood (BF) and crevicular fluid (PICF) allows us to see that this interleukin does not exist in large quantities in a healthy individual and that its rise indicates a state of inflammation / pathology.

When we look at the blood values (BF) in our study we can easily see that the concentration of IL-1β at the time of the first incision was 4,25 pg/ml compared to the 5,24 ± 3,91 pg/ml found in PICF for IL-1β. The conclusion is that IL-1β was not present in the blood (BF) at that time of surgery, it is an IL that appeared as a result of chronic trauma and established infection.

In other words, in a healthy individual there is no IL-1β expression in the blood fluid.

When we look for the healthy periodontal sulcus the amount of IL-1β is also only a trace in our findings.

This shows that the presence of inflammatory IL-1β does not exist in the blood or in the periodontium of a healthy individual (without periodontal disease) but is present in large concentrations in the peri-implant crevicular fluid.

Thus, the induced inflammation is only attributed to the fact that we have placed a dental implant.

The correlation between health and disease through the increase of IL-1β concentrations is not new in heart diseases and obesity amongst other symptoms and this relationship has already been clearly established. (Sampaio Fernandes et al. 2017; Iglesias-Linares et al. 2012)

In our investigation, the residual presence of this interleukin in the crevicular fluid (PCF) and in blood (BF) but not in the peri-implant fluid (PICF), indicates that the situation of osseointegration is not totally inert for the human body, particularly in the oral cavity.

In our premise that outlined the motivation for this investigation we proposed to establish a relationship between biomaterial inflammation and the clinical repercussions (marginal bone loss), which is in fact the case as, in general, there is inflammation-increasing behavior (measured as interleukin IL-1β) from

the day of implant placement until osseointegration is complete. This situation did not exist before the patient underwent implant placement.

The clinical repercussion of the presence of IL-1β in a high concentration at T2 (situation statistically superior to T0) may be marginal bone remodeling, that is the osteoclastic activation of the area and subsequent marginal bone loss.

In this study, the total marginal bone loss (MBL) found was mean 9.8 mm (if we considered bone loss in implants that did not remain exposed-MBL1) or mean 7.61mm (if we only considered bone loss the implants that had part of the thread exposed-MBL2).

This bone loss value is perfectly framed within the values of marginal bone loss found in the literature for this type of connection, and for the type of implant macrogeometry. (Cooper et al. 2017)

Vervaeke et al. (Vervaeke et al. 2014) suggest that implants with lower abutments reflecting the initial gingival thickness, lose more peri-implant bone, possibly by reestablishing the biological width. In our study, we found that when we have 2 mm of pre-existing gingival tissue we have an MBL of mean 1,2 mm and when we have 3 mm of preexisting BW the MBL drops by mean 0,6mm, a difference of almost 0,6 mm.

The formation of a biological width with bone remodeling of about one millimeter, in these types of implants (the ones used in our experimental work), demonstrates an even more optimum behavior than the assumptions of Albrektsson et al. for what constitute a successful dental implant. (T Albrektsson et al. 1981b)

We must not forget that our investigation followed a rationale that all clinical protocols are governed by as the most current up to date protocols in implantology, respecting that all the available evidence has been scrutinized and the best protocols selected.

We know that surgical procedures and implant-abutment selection have an impact on inflammation values and clinical protocols seem to interfere with cytokine expression.

Implant selection and protocols are very important nowadays and there is literature that shows the impact on bone and soft tissue levels.

Before we discuss and compare our protocol with the literature, a short description is made of our clinical protocol.

The experimental implant was a conical body type of implant to increase primary stability (Gualini et al. 2017a) ,and the implant was placed 2 mm to the subcrestal and the final abutment was placed on the day of surgery (one abutment one time), a technique that we know to be the best for the behavior of marginal bone. (Atieh et al. 2017).

Placing the final abutment on the day of the surgery allows for an initial sealing between implant platform and the abutment, minimizing bacterial infiltrate and reducing inflammatory response.

This concept was presented to us through the animal studies of Abrahamson and corroborated in clinical trials with high degrees of evidence. (I Abrahamsson et al. 1998; Atieh et al. 2017)

Studies by Kumpusamy et al. show that removing and placing the abutment interferes with the expression of IL-1β, removing it several times results in a rise in the final overall IL-1β expression (Kuppusamy et al. 2015). The abutment that stayed in place from the day of surgery had much lower indices of IL-1β expression than the control side (multiple disconnections).

In our study, all the abutments were placed on the day of surgery and all measurements were taken without removing the abutment, therefore representing the ideal inflammatory situation.

The use of a platform implant discrepancy (platform switching) was chosen for this study as it seems to decrease the indices of marginal bone loss.

The use of this type of platform macrogeometry combined with an internal hexagon is a situation that we know to be one of the best with regard to the precision and fitting of the prosthetic pieces.

This study analyze implant-abutment complex with regard to the acrylic-implant interface, the zirconia-implant and the titanium-implant, under electron microscope. In the microscopic analysis, no differences were found between the

different complexes and in all cases the microgap was never greater than 5 microns.

In this topic and as shown in table 136 we can see that the use of different implants with different connections may have a slight impact on interleukin expression (specially of IL-1β and IL6).

The procedures were performed with an internal hexagon and yielded a concentration of IL-1β after the implant had osteointegrated of 55.41 ± 49.85 pg/ml. Casado et al. in 2013 used 10 external hexagon implants and their IL-1β concentration after osseointegration was 67.51± 62.9 pg/ml (Casado et al. 2013a). Finally, Bielemann et al. used a conical connection with a value of 13.5 pg/ml making no mention of SD, but only a variation of 0 to 97 with 60 implants (Bielemann et al. 2017).

We can appreciate that the connection may have an impact on the expression of interleukin before and also after osseointegration is completed.

However, we have to take into account that the inflammatory potential of the patient/type of rehabilitation sample of each study is quite different. Thus, in the Bielemann study, the populations were totally edentulous, whereas (Bielemann et al. 2017) in our study and in that of Casado (Casado et al. 2013a), the situations are single and partial implant rehabilitations.

Another bias that may arise in the Bielemann et al. study is that the given concentrations are in (pg / ul) picograms per microliter, while in the Casado et al. study and in ours, the concentration results are in pg / ml (picograms per milliliter) (which is the most commonly accepted in the biochemical literature of concentrations). Elisa test calibration curves always report in pg / ml, so if we have to transform the concentrations in Bielemann of IL-1β of 13.5 pg / µl we get 13,500 pg / ml which does not seem to make sense.

Due to this problematic, the impact of the connection on the expression of interleukins is dubious and more controlled studies should be carried out to understand this impact more fully.

The literature tends to show us in relation to implant platform-geometries and microgaps, that the bigger the microgap the more bacteria will leak and as a result the potential for MBL will increase.

This clinical situation and this research was not based on the microbiological theory of marginal bone remodeling where bacteria appear to be the etiological factor, but rather on the theory that the immune response of the host to different alloplastic materials placed in the bone and soft tissues (gingiva) will produce more inflammation and increase MBL.

Our Work is based on the doubt voiced by Prof. Albrektson in an Editorial in 2014 (Tomas Albrektsson et al. 2014) and inspired by reading Huja. ((Emecen-Huja et al. 2013)

Albrektsson stated "*that oral implants may lose bone or even display clinical failure. However, progressive bone loss threatening implant survival is rare and limited to a percent or two of all implants followed up over 10 years or more, provided that controlled implant systems are being used by properly trained clinicians. There is very little evidence pointing to implants suffering from a defined disease entity entitled "peri-implantitis." Marginal bone loss around implants is in the great majority of cases associated with immune-osteolytic reactions. Complicating factors include patient genetic disorders, patient smoking, cement or impression material remnants in the peri-implant sulcus, bacterial contamination of the implant components and technical issues such as loose screws, mobile components or fractured materials. These reactions combine to result in cellular responses with the end result being a shift in the delicate balance between the osteoblast and the osteoclast resulting in bone resorption. However, the great majority of controlled implants display a foreign body equilibrium resulting in very high survival rates of the implants over long term of follow-up."* (Tomas Albrektsson et al. 2016)

Thus, in our study we have assumed that bacteria, as a factor, is a consequence of marginal bone loss and not the causative factor (in the majority of situations) as Albrektsson stated above.

However, as a factor that can influence the final result, we believe that the best way to perform this experiment is to eliminate the microgap completely and to use a single piece implant where implant and abutment are placed in a single piece, thus eliminating any microgap.

In our study this was not done. However, in addition to minimizing the impact, a final abutment was used, placed on the day of surgery, in an implant with a discrepant-type platform and all measurements were made without removing the abutment.

Thus, the use of platform-switch is reported in the literature as presenting better outcomes with regard to marginal bone loss in internal/external hexagon implants. (Strietzel, Neumann, and Hertel 2015b)

Taking into account all these assumptions, the formation of a sucrestal biological width of approximately 2 mm is perfectly acceptable.

Within our premise of study, we would like to evaluate the impact of the biomaterial on the formation of this biological space, comparing inflammation with the clinical repercussions (marginal bone loss).

With regard to the inflammatory index, there are statistically significant differences regarding the behavior of IL-1β in the different abutments.

Although between T0 and T2 the implants showed similar behaviors regarding the expression of IL-1β, at T2 the values between the different materials was statistically different, being lower when we placed a zirconia healing abutment 29.94 ± 54.07 pg/ml compared to a titanium 64.75 ± 55.24 pg/ml.

This difference in IL-1β expression at T2 led to a lower marginal bone loss in the zirconia than in the titanium, mean 10.48 mm as opposed to mean 8.69 mm if you consider MBL1 or mean 8.79 mm as opposed to mean 5.65 mm if you consider MBL2. From any angle, the tendency is to have at least 0,2 mm or less in mean marginal bone loss when using a final zirconia abutment as opposed to titanium. In this study, we cannot refute the null hypothesis with respect to the trend presented, in part, we believe, because a larger sample is needed.

When we chose our initial sample, we were expecting the marginal bone loss between abutments to be around 1 mm, but in fact the difference between them may be lower. In the literature, the evidence discusses these type of differences, but in most of the studies read, they, either did not use platform switching implants or there were multiple connections / disconnections. (Emecen-Huja et al. 2013)

A rigorous protocol of implant / abutment placement was followed in our protocol, using a platform switch with one-abutment one-time protocol and from this we can speculate why our MBL results were lower and why we minimized marginal bone resorption.

In this way, any impact of the biomaterial would still impact MBL by decreasing it even more. Thus, in the final analysis, our results were well below the calculated 1 mm.

In order to see a statistical significant correlation between MBL and inflammatory levels the sample size had to be increased.

To realize the impact of a 0,2 to 0,4 mm difference in marginal bone loss we would probably have had to proceed with double or triple the sample, which in this case would have been impossible for cost reasons.

Even so, we have been able to demonstrate in a clear and statistically significant way that the use of a zirconia abutment has an impact on the way IL-1β is expressed.

When we compared the total value of IL-1β with the literature we found that it is in agreement with the Panagakos study of 1996 (Panagakos et al. 2017) in which the average found for the expression of IL-1β was 59.47 pg/ml.

Although it is in agreement with the total expression of our investigation (our value for total IL-1β was 55.41 pg/ml), when separated by biomaterial, we verified that by altering the titanium rehabilitation protocol for zirconia, our concentration dropped to 29.94 pg/ml, well below the 59 pg/ml of Panagakos et al.

By looking only at the use of cad-cam we have found that this technology may have an impact on the final expression of interleukins since the concentration of IL-1β in the cad-cam acrylic was 31.44, again well below 59 pg/mm reported by Panagakgos et al.

When however, we compared only the expression of IL-1β with the titanium abutment, the result was 64.75 pg/ml, slightly above Panagakos et al. but comparable.

When we compare this value with Barwacz et al. (Barwacz et al. 2015) in 2016 we find, however, that these two values (ours and Panagakos) differ from the 46.19 pg/ml found by this author.

Casado et al. in 2013 finds an IL-1β expression value of 67.51 pg/ml which is very similar value to ours. We can conclude that based on the literature, the value of IL-1β in complexes Implants/titanium has high concentration values, with the values of Barwacz et al. in 2016 being the only exception.

By comparing the Panagakos et al. study to our own and others we can assume that titanium actually appears to activate increased IL-1β expression at established perimplant sites.

With respect to the strong performance of zirconia with 29.94 pg/ml, this value is in line with Barwacz et al. who found a similar value of 14.03 pg/ml. He also found the same value discrepancy in expression of IL-1β when a zirconia abutment is used in comparison with a titanium.

One important discussion topic is, if bacteria interferes with these results or not, and in my opinion the article of Scarano in 2004 is a good starting point for this discussion.

In his article in 2004 Scarano (Scarano et al. 2004) focused precisely on the adherence of bacteria to zirconia and he clearly states that his results demonstrate that zirconium oxide may be a suitable material for manufacturing implant abutments with a low colonization potential.

In fact, one confounding factor may be that if bacteria adhere to titanium more readily, then it will evoke a stronger inflammatory reaction.

As our study did not cover the microbiological aspect, so more studies would obviously need to be undertaken to discard this item. However, one thing we know from previously work is that, our clinical protocol was based on the evidence of the lowest bacterial concentration levels, the use of one-abutment one-time and platform switching which is a trusted method.

Speculating for a moment we could maintain that, the biological width formation around the clinical protocol used in our study leaves no room for bacterial

colonization to create situations which stimulate perimplantitis. But once again an RCT would need to be done to answer this assertion.

6.2. INFLAMMATORY LEVELS IN PICF COMPARED TO PCF

On the table 137 below are the results compared to those found in the literature on cytokine expression in periodontal crevicular fluid (PCF).

Table 137 - Comparison of periodontal Interleukin concentrations in the literature

Study	N	IL6 (pg/ml)				IL-1β(pg/ml)			
		PCF Health	PCF Path	PICF Integrated	PICF Path	PCF Health	PCF Path	PICF Integrated	PICF Path
Zirconia (ChenPhd)				4,76±13,83				29,94±54,07	
Acrilyc (chenPhd)				8,56 ±14,82				31,44 ±33,40	
Titanium (ChenPhd)				4,06 ± 7,99				64,75 ±55,24	
Overall (chenPhd)									
Overall PCF (chenPhd)		0				15,15			
Emecen-Huja 2013				0,5±0,1				14±2	
Barwacz 2016 Zirconia				4,94 ±3,73				14,03 ±14,68	
Barwacz 2016 Titanium				8,31±8,55				46.19±83,03	
Panagakos 1996								59,47 ±15,55	191,10 ±21,60
Casad0 2013								67,51 ±62,9	
Melo 2011				0.32±0.59				2.1± 2.74	
Kuppusamy 2015 final ab								57	
Kuppusamy 2015 healing abut								97	
Bielemann 2017				40,7(0-784)				13,5 (0,97	
Ata-ali 2015				0,53±0,63	0,9			21,2±24,2	58±8
Erdemir 2004 Toyman 2015			0,57±0,75						
Thunell 2010		1,71±1,23	2,41±324			74±117	28,4±36,9		
Fentoglu 2011		0,92 (0,52-2,47)				2,11 (0,54-63,49)			
Luo2011		2,49 ±2,57	49,8 5±12,96	16,758 ±8,932		14, 5±16,7	64,52 ±22,89	13,84 ±16,31	

PCF- Periodontal Crevicular Fluid, Path- Pathology, PICF – Peri-implant Crevicular Fluid

This study is in the unique position of being the first in the literature of Implantology to compare the inflammatory state of abutments on implants with different biomaterials.

When we compare the performance of interleukins by material (T, A, Z) in the peri-implantar crevicular fluid (PICF) and compare them with periodontal crevicular fluid (PCF), different conclusions can be drawn.

With regard to titanium, the results point to a statistically significant difference in comparing IL6 present in the PICF and the IL6 present in the PCF. In the titanium implant-based restorations, concentrations of PICF are higher than in teeth, not only at T0 but also consistently at T2.

There is a totally different response in zirconia biomaterial in comparison to PICF vs. PCF. The IL6 in zirconia shows higher concentrations at T0 than the PCF of the tooth, but at T2 they normalize with no statistical difference between the inflammatory expression in both teeth and implants.

Holding to these results, we can understand that, zirconia induces an inflammatory expression very similar to a natural tooth. Instead titanium behaves more like an exacerbated foreign body reaction.

In terms of this the acrylic is in an intermediate position between zirconia and titanium, but still evokes an inflammatory reaction stronger in PCF both at T0 and T2.

When we analyze the behavior of zirconia in relation to IL6 in the PICF and PCF, the total variation of the IL6 from T0 to T2 is zero, which is also the case in 0 in PCF. We can conclude from these results that there is no statistically significant variation in either fluid.

On the other hand, we have also seen that the variation of IL6 in zirconia itself does not change either showing that the placement of a zirconia abutment has no impact on the behavior of IL6 over time, maintaining an inflammatory expression statistically equal to that of a tooth with a healthy PCF.

The same result does not occur with titanium and acrylic which maintains an IL6 concentration higher than that found in a healthy PCF.

In IL-1β, when the behavior of the titanium abutment is observed at T0 (day of surgery), the concentration of this interleukin is lower than the value of PCF. These findings have a biological reason, since it is understood that on the day of surgery the probability of high concentrations of IL-1β is low, since this type of protein does not appear in the acute phase. It is thus understandable that IL-1β values in PICF (T0) are lower than the periodontal fluid, principaly because the healthy periodontal sulcus has a perfectly established balance with chronic infiltrate that increases the concentration of IL-1β.

However, at T2, titanium has a different behavior, and the concentration of IL-1β is statistically higher than the value presented by the PCF of a tooth. In this way placement of an endosseous implant with a titanium abutment induces stronger inflammation than the value of PCF.

However, once again zirconia exhibits a distinct behavior when we compare IL-1β of PICF with PCF. At T0 the concentration of IL-1β in zirconia is lower than the concentration of IL-1β present in PCF, but unlike titanium the concentration of IL-1β at T2 is similar to that of PCF and not higher.

Thus, with respect to the behavior of zirconia in relation to the concentrations of IL-1β and IL6, the behavior is very similar to the PCF of a healthy natural tooth.

The acrylic with respect to IL-1β is closer in behavior to the zirconia abutment, but with respect to the concentration of IL6 it resembles the titanium more.

When we compare crevicular fluid levels obtained with those found in the literature we observe the following:

Regarding the total of IL-1β and IL6 levels present in the periodontal fluid (PCF), the results show that at T0 there is an absence of IL6 in the sulcus, which is in line with Luo's findings in 2011. (Luo et al. 2011)

Thunnel in 2015 also observed, only traces of this interleukin in crevicular fluid in the healthy periodontium. (Thunell et al. 2010).

Fentoglu in 2011, found only traces of IL6, which confirms the idea that IL6 is not present in a chronic established infiltrate, as is the case of the periodontal sulcus. (Fentoğlu et al. 2012)

As for IL-1β, the scenario is totally different. In our study, the amount of IL-1β found in the periodontal sulcus was 15.15 pg/ml, which is in line with Luo et al. 2011 and Fentoglu in 2011, but not with Thunnel in 2010 which is slightly higher.

Thus, it becomes clear that in case of periodontal health there is an absence (or at least residual) IL6 in the gingival sulcus, a distinct scenario with respect to IL-1β which seems to be present in higher levels, partly due to the constant promotion of bacteria.

Panagakos et al. show that when equilibrium tends towards inflammation/ infection, as is the case of perimplantitis, IL-1β as an acute phase messenger rises in concentration to become a signaling molecule.

There were similar results for IL6, but more moderate with regard to the increase in values in cases of infection.

Luo et al. in 2011 confirms the previous results regarding the inflammatory response measured in IL6 and IL-1β, that in cases of periodontal infection the values go up 10 to 15 x its concentration.

It can be seen from the comparison of studies that the tendency for peri-implant infection is to show a smoother rise, while the infections of periodontal nature show more abrupt rises in basal IL values.

There are two main reasons for this. Firstly, looking at the works presented in table 138 we can see the baseline values of IL-1β present in the PICF are higher than the PCF, leading to the conclusion that there is a state of permanent inflammation when an implant is placed in bone and periodontium which is, in general, more intense than the PCF.

Secondly, the foreign body reaction seems to be present when we place dental implants (either at baseline T0 or at T2), since the concentrations present in peri-implant fluid are higher than the ones in the periodontium.

Table 138 - Comparison of Blood/Serum Interleukin concentrations in the literature.

	Blood	IL-1β	IL6
Fentoglu 2011	serum	2,94 (0,80-26.08	5,82 (3.51-62,53)
Chen Phd 2017	serum	4,25	0

6.3. SECTION 5.5 MARGINAL BONE LOSS (MBL) AND INFLAMMATORY PARAMETERS DISCUSSION

Placing an implant in bone has an impact on the host's biological system, an alloplastic material producing a response that in the case of dental implants may be translated into marginal bone loss.

The theory behind this clinical feature, is that placement of an implant together with its final crown, creates an interface (microgap) between the crown platform and the implant that will always be a focus of bacterial contamination. (I Abrahamsson et al. 2017)

Depending on the connection type, different grades of contaminations will arise.

There are several factors that affect the number of bacteria present, and are normally correlated with platform geometry, surgical technique or host factors.

Many important issues arise when it comes to surgical technique, but the apico-coronal position of an implant is the one that most consistently interferes with the bone resorption pattern.

If we place an implant at any bone level, there will be contamination of the microgap that will eventually lead to a physiological host response based on inflammation, which attempts to neutralize this harmful stimulus.

The equicrestal position of the implant induces a direct contact of the microgap against crestal bone, inducing a reformulation of the biological width, normally 2,5 to 3 mm below the original crestal bone margin (Blanco et al. 2012). The supracrestal position positions the microgap away from marginal bone, creating a more favorable environment. (Romanos et al. 2015)

The clinical impact of this inflammation may be marginal bone remodeling which eventually leads to resorption.

It is evident in the literature that values of MBL are higherer with the supracrestal position against the crestal position of an implant, the values of -0,03 mm MBL vs. -0,52 mm (Hermann et al. 1997), the values of -1,13 mm vs. -0,38mm (Cochran et al. 2009),and -0,81 vs. -1,23 (Veis et al. 2010) show this to be the case.

The contact of a titanium alloplastic material with the host connective tissue of the periodontium can lead to an inflammatory phenomenon foreign body reaction.

It is precisely from this point of view that the assertions of our thesis are reliable. The correlation would be that the more inert the biomaterial is, the less inflammation it will produce, thus giving rise to less MBL.

Traditionally the attribution of a noxious stimulus created by the existence of a microgap (implant-crown interface), would lead to the explanation that bacterial colonization initiates an inflammatory reaction of the body to the infectious stimulus.

But bacterial colonization may also be a secondary opportunistic colonization followed by an initial host immune response as stated by Albrektsson.

The consequences may be the same. In the bacterial theory of marginal bone remodeling, bacteria get their first and only boost after the inflammatory reaction responds. In the biochemical theory, the bacteria come after the body responds with inflammation to the auto-immune response triggered by insertion of an implant. In other words, we have the same consequence - inflammation - but very different etiologies.

Inflammation is comprised of numerous inflammatory mediators that are released into the crevicular medium such as IL-1β, IL6, arachidonic acid, some platelets and platelet deriving factors.

After initiation of a typical inflammatory reaction, many inflammatory proteins and potent osteoclast activators are triggered causing marginal bone resorption.

The level of implant placement (equicrestal, subcrestal or supra crestal) conditions the expected marginal bone resorption, as seen in table 138.

Another platform factor that may have an impact on MBL is the platform collar with regard to the biomaterial used.

Traditionally, Brånemark implants were designed with a 1.8 mm polished collar, so this electropolished titanium surface would be in contact with soft tissues thereby promoting adhesion between the hemidesmossomes of the epithelium and the surface of the implant platform. The same phenomenon occurred with the implant designed by Schroeder in which the polished collar would be 2.8 mm, a little higher to the external hexagon of Brånemark. Notice that this last implant had an internal connection.

Current implants display rough surface platforms, in contrast to the originals, although they have several benefits in early loading. It is in late failure that concerns arise due to perimplantitis.

In our study, we decided to measure the marginal bone loss in two distinct ways that we called MBL1 and MBL2.

The MBL1 measurement consisted in measuring all the marginal bone loss that an implant suffered from T0 to T2. That is, an implant placed 2 mm subcrestally would have 2 mm of supracrestal bone at the implant platform at T0. If at T2, bone loss was 1mm, it would mean that we would still have a 1 mm of bone above the platform if the implant had not been exposed.

In a number of publications, this value would be considered 0 or as had been no marginal bone loss, the implant did not lose bone below the implant platform.

In positive bone loss (when the level of bone remodeling passes below implant platform), the reported result was a mean MBL 1 of -0,98 mm.

In the MBL2 measurement, this is precisely equated with commencement of measuring marginal bone loss from the moment the bone is at the platform level (to the value 0) or below and there will be negative values.

The impact of our study shows an implant that does not have the platform exposed is an implant that does not have titanium biochemical expression and

as such, cannot affect the expression of interleukins and lead to a skewed value.

On the other hand, it is important to know if biomaterials have any influence on bone remodeling and what percentage of this intense remodeling leads to implant exposure. The result achieved in our study was -0,761 mm in the overall pool of implants.

In both MBl1 and MBL2 the mean MBL achieved are in line with the best performances of different connections at different bone levels.

When we compare this to the conical connection several studies show similar values and outcomes. (Schwarz, Hegewald, and Becker 2014).

The results are comparable to the subcrestal approach but are worse when we compare them with dental implants in a supracrestal approach. The 0,16 mm reported by Siadat (Siadat et al. 2012b) and the 0,54 mm by Cordaro (Cordaro, Torsello, and Roccuzzo 2009) with the SLA surface and the tissue level implant are unsurpassed in the literature.

In fact, when the results are broken down by biomaterial, we see that the zirconia abutment shows a tendency to reduce marginal bone loss almost to the level of the supracrestal implants which can indicate a better approach.

Coincidentally, the titanium and acrylic abutment material reported the highest amount of mean MBL.

With regard to marginal bone loss, in our study we failed to establish a correlation between increased inflammatory expression and increased marginal bone loss, despite the tendency being evident.

The complex implant-zirconia abutment presented in our study showed a statistically significant difference in the expression of IL-1β and presented the best levels of bone loss. The only underlying truth is that the difference of MBL for the acrylic and titanium abutments were not statistically significant.

However, it is the opinion of the authors that if the sample was larger, the difference was enough to claim that there is a statistically significant relation in the performance of the zirconia abutment in terms of inflammatory expression.

The mean marginal bone loss between the acrylic and titanium abutments was similar, as was the expression of interleukins.

In fact, the combination of placing a 2 mm subcrestal implant with a platform switching implant and a zirconia healing abutment on the same day of surgery has been proven in our study to be the best therapeutic option, with an overall lower expression of IL-1β and IL6 and less MBL than the titanium or acrylic options.

Titanium at T2 exhibits a total IL concentration of 64.75 ± 55.24 pg/mm while Z presents values of 29.94 ± 54.07 pg/ml and acrylic 31.44 ± 33.40 pg/ml, to Z almost three times less than the T and the A almost half of the T.

Table 139 demonstrates the impact that the placement to different levels of bone can have on marginal bone resorption.

Table 139 - Comparison of Implant Placement at different bone levels and the impact on marginal bone loss (MBL) – Why choose a subcrestal position?

Article	Crestal level	MBL	Study	Connection
Jung 2008	+1mm	-0,17	V	PS, S, CM,
	0	-0,15		
	-1mm	-1,32		
Cochran 2009	+1	-0,38	V	PS, Ns, CM,
	0	-1,13		
	-1	-0,19		
Barros 2010	0	-0,58	A	PS, Ns, CM
	-1,5	-0,14		PS, Ns, CM
Hammarle 1996	-1mm	-2,2		NS, I
	0	-1,02		
Weng 2008	0	-0,23	H	CM, PS,Ns,RS
		-0,51		EXT, Rs
	-1,5	+0,19		CM, PS,Ns,RS
		-0,57		EXT, Rs
Romanos 2015	≥0,5	-1,84±1,31	H	CM, Ns
	≤0,5	-1,41±1,65		
Palaska 2016	Subcrestal	-0.68	H	NS ,I,PS

	crestal	-0.79		NS ,I,PS
	Subcrestal	-0.49		CM ,PS,Rs
	Crestal	-0.40		CM ,PS,Rs
Hermann 2011	Supracrestal 2mm	-1.28 ± 0.21	V	
		-1.11± 0.27		
	Equicrestal	-0.52 ± 0.40		
		-0.43 ±0.43		
	Supracrestal 3mm	+0.16 ± 0.40		
		-0.03 – 0.48		
Kutan 2015	-1	-1.21±1.05	H	PS,Rs
	Equicrestal	-0.56±0.35		PS,Rs
Veis 2010	EquiCrestal	-1,23	H	EXT,RS,S,PS
	SupraCrestal	-0,60		EXT,RS,S,PS
	Subcrestal	-0,81		Ext,RS,S,PS
	EquiCrestal	-1,13		Ext,RS,S,PM
	SupraCrestal	-0,69		Ext,RS,S,PM
	Subcrestal	-0,039		Ext,RS,S,PM
Gualini 2017	0,5 Subcrestal	-0.21 ± 0.51	H	S,NS,Rs
	1,5 Subcrestal	-0.11 ± 0.36		S,NS,Rs
Val 2017	Subcrestal	-3.42± 2.31	V	CM, Rs
	Crestal	-4.44± 1.03		CM, Rs
	Subcrestal	-3.38± 0.41		CM, Rs
	Crestal	-4.01 ±0.18		CM, Rs
de Siqueira 2016	Equicrestal	-1.03± 0.60	H	CM, RS
	Subcrestal	-0.66 ±0.38		CM, RS
Degidi2016.	Sucrestal (1-3mm)	-0.42 ± 0.77	H	CM,RS
Chen Phd	Subcrestal (overall)	-0,761	H	I,Ns,OS
	T Subcrestal	0,88		
	A subcrestal	0,87		
	Z subcrestal	0,56		

PS- Platform Switch, PM-Platform Matched, CM-Cone Morse, RS-Rough Surface, Ns-Non-Submerged, S-Submerged,EXT- External Hex, I-Internal Connection,V-Animal Study, H-Human Study

Our results showed that a rough surface collar platform switching implant placed subcrestally and without removing the abutment that was placed on the day of surgery had a mean MBL of -0.761mm.

When we compare our results with subcrestal placement in similar human studies we see that the RCT by Gualini et al. in 2017, with rough surface in a non-submerged clinical situation, reported an average MBL of -0.21 ± 0.51 mm concluding in this high level of evidence, that there were no statistical or clinical differences when placing implants 0.5 mm or 1.5 mm subcrestally. Therefore, according to this study, clinicians can place implants in the interval between 1,5 or 0,5 below the crest and the clinical result will be similar. (Gualini et al. 2017b)

Another study by Veis et al. with the external hexagon, platform switch and rough surface collar, similar to our study, found a mean MBL of -0,81mm when using a subcrestal approach compared to the crestal placement of the implant-abutment connection. The latter resulted in higher marginal bone resorption in both straight and platform-switched abutments. (Veis et al. 2010).

From these two RCT studies we can conclude that the subcrestal position of the implant is better than the crestal position in terms of MBL.

These results are in line with our study proving that we can decrease the Albrektsson success criteria by almost 1,5mm.

Palaska et al. with an internal connection and rough surface implants found a mean average MBL of - 0.49 mm, a lot less than ours and the Veis study.

Interestingly, the author concluded that, in relation to the alveolar bone level, the connection between fixture/abutment rather than the vertical implant placement, seems to affect peri-implant marginal bone resorption. (Palaska et al. 2016) ,

In contrast to Palaska, Kutan et al. found a mean MBL of -1.21 ± 1.05 mm, almost 0,5 mm more than our study, and clearly states that the randomized clinical trial confirmed the hypothesis that placing platform-switching implants 1 mm below bone level reduced marginal bone loss. The author also concludes that for reduce bone resorption, platform-switching implants should be placed below bone level. (Kütan et al. 2015)

Romanos et al. in a 2015 article goes against the fact that the implant should be always be placed subcrestaly. In his research with the cone morse Ankylos implant, he found that subcrestal or crestal implant placement in combination with delayed loading was associated with similar initial implant stability and subsequent crestal bone loss of -1,41 ± 1,65 mm and a subcrestal value of -1,81 ± 1,31 mm. (Romanos et al. 2015).

Table 140 - Comparison of Implant Placement with different surgical techniques (One Connection (ND) vs. multiple disconnection (MD)) and the impact on marginal bone loss (MBL) – Why choose to read the II levels without removing the abutment?

Study	MBL Disconnection	MBL No disconnection
Iglhaut 2013	1.66 ± 1.26	. 0.95 ± 0.5
Rodriguez 2013.	1.09±0.25	
Canullo 2010	0,43±0,12	0,33±0,08
Degidi et al. 2014	0,17±0,21	0,13±0,22
Grandi et al. 2012	0,44±0,03	0,09±0,03
Koutouzis 2013	0,28±0,16	0,13±0,20
Luongo et al. 2015	0,09±0,20	0,08±0,16
Molina et al. 2016	0,32±0,58	0,01±0,54
Chen Phd		0,761
Chen Phd Zirconia		0,56

Another factor that affects marginal bone loss is the fact that the abutment is disconnected multiple times.

Our study was designed to place one abutment at the time of surgery and never take it off. When compared to the literature, our marginal bone loss was higher than the other clinical assays, but the values are closer together when we use the zirconia abutment as seen in table 140.

Our result was –0,56 mm of MBL with the internal hex using a platform switch in a one-abutment one-type situation. The best report on zero disconnection is from Molina et al. with -0,01 ± 0,54 mm (Molina et al. 2017) in which he concluded that the connection and disconnection of healing abutments is

associated with significantly increased bone loss during the healing period between implant placement and 6 months post-loading.

In the Luongo et al. study no strong correlation between connection and disconnection of the abutment and the impact on marginal bone loss was found. Luongo's preliminary short-term data (4-month post-loading) showed that repeated abutment changes do not alter bone levels significantly, therefore contrasting with all the other literature. (Luongo et al. 2015)

The Grandi et al. study again shows a tendency for better results when not taking the abutment off ,(Grandi et al. 2012) and the author concludes that the non-removal of abutments placed at the time of surgery can result in a statistically significant reduction of the crestal bone resorption around the immediately restored implants in cases of partial edentulism. However, a difference of 0.3 mm may not have a clinical impact.

Degidi et al. supported these findings and stated that the non-removal of abutments placed at the time of surgery improves the stability of healed soft and hard tissues around the immediately restored, subcrestally placed tapered single maxillary implant. (Degidi et al. 2014).

Due to this evidence, we chose to utilize this clinical situation, although the results were higher that rest of the literature.

Table 141 - Comparison Implant Placement with different surgical techniques (submerged and non-submerged) and the impact on marginal bone loss (MBL) – Why choose a Non-Submerged Approach?

Article	Study	Connection	Microgeometry (surface)	Surgical technique	MBL
Engquist 2005	H	External	Machined	SM	-1,89±0,06
		External	Anodized	SM	-1,79±0,07
Crespi 2007	H	External	Acid.etch	SM	-1,16±0,51
Cordaro 2009	H	Internal	SLA	1 stage	-0,54±0,33
Cehreli 2010	H	External	Anodized	1 stage	-1,21±0,10

		Internal	SLA	1 stage	-0,73±0,06
Enkling 2011	H	External	SLA	1 stage	0.47±0.46
Tallarico 2016	H	External	Anodized	1 stage	-0,87±0,45
Hammarle 2015	H	internal	SLA	1 stage	-0,47±0,64
Sanz 2015	H	Internal	SLA	1stage	-0,68±0,98
Shibly 2016	H	external	Anodized	1 Stage	+0,75±0,17
Siadat 2012	H	internal	Anodized	1Stage	-0,16±0,3
Cordaro 2013	H	internal	SLA	1 stage	-0,54±0,76
Nader 2016	H	Internal	SLA	1 Stage	-0.29 ± 0.36
Chen Phd	H	Internal	SLA	1 Stage	0,761
Chen Phd zirconia	H	Internal	SLA	1 Stage	0,56

Table 141 represents the impact on MBL when you decide to submerge an implant.

The -0,761 mm in our results can be compared to the internal connection non-submerged work of Cordaro (Cordaro, Torsello, and Roccuzzo 2009) which reported -0,54±0,33 mm and Cehreli who reported-1,21±0,10 mm. (Çehreli et al. 2010b).

Table 142 - Comparison Implant of Placement with different surgical techniques (Platform Switch v.s Platform Matched) and the impact on marginal bone loss (MBL) – Why choose a Platform Switch Implant?

Type of study	MBL with PS	MBL no PS
Strietzel 2015	0.49	1.01 mm
Hsu 2017	0.36 ± 0.15	
Chen Phd	0,76 ± 0.2	

6.4. SECTION 2.6 DISCUSSION ON BIOLOGICAL WIDTH FORMATION AND CORRELATION WITH MARGINAL BONE LOSS AND INFLAMMATORY LEVELS

Table 143 - Comparison of Biological Width formation and the impact on marginal bone loss (MBL)

	Type study	Height tissue(mm)	Position	MBL	surface
Judgar 2014	human	3.26 ± 0.15	Subcrestal		
		2.55 ± 0.16			
Negri 2014	dog	3.34 ± 0.53	Subcrestal	2.05 ± 0.36	
Huh 2014	Dog	2.88± 0.66		1.49 ±0.55	M
		2.36 ±0.63		0.60 ±0.38	M
		3.18 ±0.63		1.15 ±0.56	Rs
Cohcran 2007	dog	2.33±0.729	Subcrestal 1mm	0.232±0.216	Rs, PS
		1.77±0.340	Supracrestal1mm	0.966±0.672	PS ,Rs
		1.97±0.531	Equicrestal	0.30 ±0.196	PS, Rs
Linares 2015	minpig	2,02±0,88	Supracrestal		T, Mc
		2,12±0,35	Supracrestal		T,ACC
		2,14±0,46	Supracrestal		T,Zc.
Blanco 2012	dog	4.01±0,64	Supracrestal		Pc
		3.9±0,64	Supracrestal		Pc
Vervaeke2014	human	Less 2(ab height)		1.17	Rs
		2mm (ab height)		0.86	Rs
		3mm (ab height)		0.38	Rs
Blanco 2010	dog	3,02			Pc
		3,69			Pc
Mareque2014	dog	3.20±0.78			Rs
Negri 2015		3.44 ± 0.47			Mc

		2.99 ± 0.53			Mc
Blanco 2016	dog	3.01 ± 0.44	Subcrestal		Rs, Z ab
		3.18 ± 0.47	Subcrestal		Rs, T ab
Chen Phd	human	2mm measure		12,04±12,28	Rs
Chen Phd		3mm measure		6,40 ± 8,59	Rs

M-machined surface Rs- Rough Surface, T-Titanium ,Z-Zirconia, Ab-Abutment,Pc-Polished collar, Zc-Zirconia Colar, ACC-Acid-etch collar, Ps-Platform Switch

The formation of the biological width is undoubtedly one of my favorite parts of this thesis. In measuring the amount of gingival tissue that covered the edentulous area at the time of implant placement (T0), our results demonstrated that the height varies between 2 to 3 mm of residual gingiva and in exceptional situations 1 or 4 mm was found in very thin or very thick biotypes.

In statistical terms, these exceptional results of 1 and 4 mm did not enter into our statistical analysis, since the sample was too small to be taken into consideration.

Following, marginal bone loss and inflammatory infiltrate was measured in terms of IL-1β and IL6.

At T0 we obtained a statistically significant difference in IL expression, both at IL-1β and IL6, where the expression was higher in 3 mm of residual gingiva.

A concentration of IL6 at T0 of 7.41 pg/ml was obtained with 3 mm of gingival tissue, and with 2 mm, 2.87 pg/ml.

At T2 the values were 9.67 pg/ml and 4,36 pg/ml for the 2 and 3 mm of residual gengiva, respectively.

In IL-1β in 3 mm, the values at T0 were in 5.5 pg/ml, with 2 mm, 4.25 pg/ml and at T2, 46,67 pg/ml and 38,76 pg/ml, respectively for the 2 and 3 mm residual tissue.

In other words, at T0 the inflammatory expression presents a statistically significant correlation: on the day of surgery the preexisting height of the gingiva affects the inflammatory reaction and the expression of IL in the area, which is less gingival height, less inflammatory reaction, in the expression of IL-1β and IL6.

At T2 this value is no longer correlated, that is, 2 or 3 mm of residual gingiva is not a predictor of MBL or inflammatory infiltrate.

These results may make sense, since at T2 there is already a specific, chronic inflammatory reaction resulting from having an alloplastic material producing a foreign body reaction. This no longer depends as much on the amount of blood vessels or potential inflammation, as it does on the response to acute trauma.

Once again, the study of the biochemical perimplantar dynamics becomes very interesting since almost all the studies of table 143 provides a histomorphometric perspective, showing us the histological architecture of the tissue but not the dynamics of these tissues in relation to the cellular biology and expression of cytokines.

This study is one of the first to relate the initial gingival height and the impact that this height has on the inflammatory reaction and marginal bone loss.

As we have already seen, there is a tendency for there to be more marginal bone loss when in 2 mm of tissue and less MBL when we have 3 mm, although the correlation is not statistically significant.

The tendency of the body to show a stronger capacity for resorption when there is less residual gingiva can be explained in light of the studies of biological width formation.

If we observe closely in the table 143 almost all the articles report the formation of a biological space of 3 or more mm, although in most cases the studies are in an animal model.

When we started with 3 mm of residual gingiva, the periodontium had less to reformulate since 3 mm already existed, unlike in the initial 2 mm that for the biological width to form where the periodontium had to reabsorb (marginal bone) to be able to form a biological space of 3 mm.

This may be an explanation which is speculative but given the nature of the facts may be the actual truth.

These results of marginal bone loss are in line with Cochran et al. studies where resorption phenomena were found in smaller in small biological spaces, 2.33 ± 0.729 mm for a MBL 0.232 ± 0.216 mm and higher values 3.18 ±0.63 mm less MBL 0.30 ± 0.196 mm (Cochran et al. 2017). Also, in a study by Vervaeke et al. a 2 mm biological width height of 1,17 mm of MBL and with 3mm BW, was reported with a corresponding MBL of 0,38 mm, suggesting that implants with lower abutments which reflect the initial gingival thickness, lose more peri-implant bone, possibly by means of a re-establishment of the biological width (Vervaeke et al. 2014).

Looking at the groundbreaking work of Blanco and coworkers we see that independent of the surgical technique, the BW is always approximately 3 to 3,5 mm depending on the study. (Mareque et al. 2014; Blanco et al. 2010; Liñares et al. 2015; Blanco et al. 2012, 2016b)

We hypothesize that the biological width in humans may be a slightly less, and this is very important since all implant designs are made based on the assumption of the 3 to 3,5 mm biological width formations. In fact, several histologic studies regarding peri-implant soft tissues and biological width around dental implants have been done in animals.

However, these findings in human peri-implant soft tissues are very scarce.

The only human study on BW in humans, is the Judgar et al. study (Judgar et al. 2014), where the biologic width dimension ranged between 2.55 ± 0.16 and 3.26 ± 0.15 in one- and two-piece implants, respectively. This difference was influenced by the connective tissue attachment, while sulcus depth and epithelial junction showed the same dimension for both groups.

In other words, when implant clinicians use everything at their disposal to eliminate the microgap and enhance tissue biology, the BW moves in the direction of 2,5 mm and not 3,5 mm.

If we look closely, the shorter BW width found in the Judgar et al. study was achieved by decreasing the connective tissue attachment and not the epithelium, which could be why the area was less inflamed. If there had been

more inflammation of the area (as he found on the 2-piece implants) then the connective tissue height would be greater, reflecting the BW formation, which would in turn be greater.

We emphasized that more RCT on this matter should be undertaken because the construction of a 2,5-mm machined collar on an implant is very different to a 3,5 one when it is not needed.

I personally think that the answer to the impact of dental implants on marginal bone is in this delicate balance between molecular signaling and biological response to biomaterials despite the presence or absence of bacteria.

It is well proven in the Judgar et al. study that even by eliminating the microgap as one does with a one-piece implant, there is still, in the absence of bacteria, marginal bone remodeling and formations at different levels.

6.5. SECTION 5.7, 5.8, 5.9 SECONDARY OUTCOME MEASURES DISCUSSION ON THE IMPACT OF AGE, GENDER AND ANATOMICAL POSITION ON MBL AND INFLAMMATION

It is important to note that the study design did not set out to find statistical differences in the secondary outcomes, but nevertheless the results are interesting.

When it comes to age, the only significant difference that we found was at T0, where IL6 differs significantly with age and, on average, is significantly higher at ≥ 65 years. The same conclusions can be drawn for IL-1β and in total, showing that at T0 patients older or equal to 65 years old tend to express more inflammation (IL6/ IL-1β) at early stages of implant placement, than patients below 65.

Becker et al. in 2016 (Becker et al. 2016) had already reported a minimal impact on bone resorption, maintained that patients over 70 years old who received dental implants had excellent implant survival rates, low periodontal disease index scores with minimal changes in interproximal bone levels, but our study is the first to compare IL-1β /IL6 with age, making our results unique.

As far as age is concerned, it is perfectly understandable that alveolar

remodeling is slower. The metabolism throughout the body is very different at a young age. The fact that people over 65 years exhibit a more exuberant inflammatory reaction may be of great importance in fields such as therapeutics and pharmacology since, in knowing this, we can attenuate the inflammation by targeting IL with the appropriate medication.

There is obviously room for broader speculation around this particular area.

And if we talk about gender, the most important parameter was also at T0, where IL6 was, on average, significantly higher in males than in females. But despite this the inflammation rate measured in IL6 was higher where more implants were exposed (MBL2) in females. This showed us that the worst combination possible in terms of IL6 expression would be a female over 65 years that needed an implant in the maxilla.

The results by anatomical position showed clearly that MBL2 differs significantly with the position, and in the maxilla, bone loss is, on average, significantly higher.

It goes without saying that, this data has limited clinical power for recommendation since, despite being this being a randomized clinical control trial, it was not the intention to study this secondary outcome in which a lot of confounding factors can affect the results.

We do not recommend extracting any clinical recommendation from these secondary outcomes but there is obviously a tendency that we must be aware of and it can also serve as the starting point for new investigations in the field.

There is no positive relationship between inflammation and MBL in all the items studied (age, gender, anatomical position).

When we state that implants can lose more bone just because they were inserted in the maxilla over the mandible is not a new feature, in fact Naert in 2002 reported the same findings, that implants in the maxilla tended to lose more bone that implants in the mandible.

But the literature is limited when trying to research these topics, so there is still a pressing need for more RCT.

Table 144 - Literature on Age, gender and the impact on inflammation rate

Author	Age	Gender	Position
Negri 2014	Mbl 50(60	No difference	Maxilla 0.9 ± 0.1mm max 0.7 ± 0.2 mand.
Becker 2016	Young –0.4 mm Old –0.1 mm		
Naert 2002			Higher in the Maxilla s 0.31 mm/year and after that 0.015 mm/year

6.6. SECTION 5.10 DISCUSSION OF THE TIME OF SURGERY, INFLAMMATORY LEVELS AND MBL.

In terms of the duration of the surgical procedure no correlation was found between time of surgery and inflammatory rates or MBL.

We could speculate that this was due to the fact that they were all single implants, the overall surgery time was very fast for all of them and

that if surgery time was increased the inflammatory reaction would probably have been higher.

6.7. SECTION 2.11 DISCUSSION OF PRIMARY STABILITY, INFLAMMATORY LEVELS AND MBL.

The table shows evidence based, high level RCT articles on primary stability. They are all human RCT studies, that showed different primary stability results.

Table 145 - Comparison of Primary stability in different studies.

Author	Implant	ISQ T1	Mand	Mx	Age	Male	Fem	ISQ T2
Lages 2017		88.27 ±5.70	Y		52,3			
Aksoy 2009	Zimmer (swiss)	72,28	71,33	74,36	46,28	70,2	77,63	
Bergkvist 2017	Straumann (tissue level)		66,5	51,6	70,1			
Merheb 2010	Straumann	67,98						
Pagliani 2012	Neoss	75	Y posterior	Y posterior				
Turkylmaz 2008	Nobel MkIII	65,7				67,3	64	
Waechter 2017	cylinder	70.87 (67.94)						
	Tapered	73.58 (68.58)						68.0 ±5.5
Gehrke 2015	conical	65.8 ±6.22						
	Semi conical	63.6 ± 5.95						67.0 ± 5.7
Chen Phd	Tapered Biomet T3	61,3 ±13,04						67,47 ±6,52
		63,95 ±6,78						70,08 ±5,78
		63,12 ±10,3						65,7 ±5,71

In our study, we found that there are significant differences in stability between T0 and T2, and, on average, stability is significantly higher at T2 than at T0.

Our average stability measurements were 61,3 ± 13,04 ISQ for the titanium, 63,95 ± 6,78 ISQ for the acrylic and 63,12 ± 10 for ISQ for the zirconia healing abutments.

On average, this is in line with the literature on this topic. Gehrke in a resonance frequency analysis–based randomized split-mouth clinical trial achieved similar ISQ values with a conical implant (65,8 ± 6,22 ISQ) and with a semi-conical implant of 63,6 ± 5,95 ISQ values, all of which were in the maxillary arch. (Gehrke, da Silva, and Del Fabbro 2015)

When comparing in the anatomical position in our study, we found that at T0, stability differs with position, being on average, significantly higher in the mandible than in the maxilla (66,44 ± 7,10 ISQ compared 66,44 ± 7,10 ISQ). The results are in agreement when we compare them to the studies of Aksoy et al. (Aksoy, Eratalay, and Tözüm 2009) and Bergkvist et al. (Bergkvist et al. 2017) A mean average of 74,36 ISQ values were found in the mandible in Aksoy et al and an average of 51,6 ISQ in the maxilla and 66,5 ISQ in the mandible were found in Bergkvist et al. In other words, the primary stability was higher in the mandible than in the maxilla, although at T2 in our study there were no statistical differences between the maxilla and mandible (66,56 ± 6,62 ISQ vs 69,53 ± 4,98 ISQ). This is also understandable since at T2, we are already talking about osseointegration and not primary stability.

It is biologically plausible that stability measures are higher in the mandible than in maxilla since in the majority of cases the maxilla has the tendency to have softer bone, thus increasing torque values and implant stability measurements.

For example, in comparing the posterior mandible with the posterior maxilla it is known that the first is mainly comprised of type III bone (meaning a good cortical plate and trabecular spongy bone), while the second is mostly made up of type IV bone.

In the mandible, this cortical plate is an “anchor” that enables the implant to achieve higher torque values than the opposite side of maxilla.

Our results demonstrate this, and the ISQ values were statistically different between them.

Some authors have achieved higher stability values by altering implant microgeometry. Pagliani et al. with the neoss ® implant achieved 75 ISQ torque values and Waecheter by undersizing the osteotomy torque values of 70 ISQ for a cylinder implant and a 73 ISQ values for a tapered implant, concluded that bone site characteristics can influence insertion torque and implant stability (Pagliani et al. 2012b)(Waechter et al. 2017).

In the work of Lages et al. where torque reached higher levels in the mandible, an average of 88,27 ISQ values was used. Lages maintains that ISQ values should only be measured directly to the implant and not to the intermediate abutment which produces different results. (Lages et al. 2017)

One of the first conclusions regarding marginal bone remodeling is that at T0 (baseline) and T2, implant stability is not significantly related to marginal bone loss (either MBL1 or MBL2), nor is it related to inflammation, namely to IL6, IL-1β and in total.

This makes sense as marginal bone resorption is a multifactorial parameter, influenced by a number of factors. Although there are cases of high torque evoking resorption rates, research by Trisi shows that there are no correlations between torque values and different bone remodeling patterns. (Trisi et al. 2017)

Systematic reviews also show that torque is not correlated to marginal bone loss. In research by Berardini et al. no significant difference in marginal bone resorption and implant failure rate between implants inserted with high or low insertion torque values was shown. (Berardini et al. 2016)

However, there may be a threshold of primary stability that has to be respected beyond which an implant can be lost or present higher MBL than implants placed within the normal range of torque.

ISQ levels clearly indicate that the interval between 50 and 80 ISQ is more than acceptable for primary stability, and our values never exceeded these limits. Therefore, MBL was not affected by this particular parameter as

ISQ levels beyond this range can interfere with MBL.

In terms of primary stability and osseointegration measures, there is no evidence in our study of this being very important when we compare them with inflammatory levels.

CHAPTER 7. FINAL REMARKS AND CONCLUSION

Conclusions

In our RCT (which is considered to be highest level of evidence) titanium exhibited the most inflammatory behavior compared to acrylic and was statistically very different from zirconia, when we consider, IL-1β inflammatory expression in particular.

The objective of this Phd was to understand how IL responded to dental implant placement.

The main thrust of the research was to understand the biochemical underpinnings of osseointegration, which is why we studied the inflammatory reaction based on IL-1β and IL6, two of the most potent IL expressions discussed in the periodontal literature.

In addition, we wanted to know how biomaterials placed over titanium dental implants affect the healing pattern.

For this purpose, we chose the 3 most commonly used materials in dental implantology, acrylic, zirconia and titanium.

From the literature, we know that an implant osseointegrates in bone and after osseointegration healing, there are only remodeling patterns, but the implant bone complex remains inert in some way.

It is in the soft tissue that the healing pattern varies over time, mainly because the connective tissue has the ability to respond to foreign body reaction.

There is a trend in the implant dentistry, whereby one of the causes of MBL is seen as arising from an imbalance in the host-foreign body reaction, which translates into inflammation, where bacteria come second contrasting to current theories where infection comes first.

The clinical implant protocols have been established according to the gold standard described in the literature as of October 2017 with

one abutment placed once in a 2 mm subcrestal platform switch implant.

We wanted to have a study that produced a clinical recommendation for all practitioners and we knew that an animal study alone couldn’t provide all the answers.

Bearing this in mind, a Randomized Clinical Control Trial (RCT) was undertaken with a reliable sample selection protocol.

The study was registered on the online platform of Clinicaltrials.gov to avoid wrong or distorted conclusions, giving the study an authoritative identity.

The animal study was undertaken first, for 3 main reasons: 1. optimization of the extraction cytokine methodology 2. for sample size calculation of the RCT study, and 3.- to optimize the Elisa reading and cytokine extraction methods.

For the overall inflammation pattern, independent of the biomaterial used we found no significant differences in the overall Interleukin variation in IL6 between T0 and T2, for IL-1β and in total (IL-1β +IL6). We also found that the difference is significantly higher at T2, showing that there was an increase IL concentration from T0 to T2.

When inflammation was analyzed at each time frame and by biomaterial, at T0, IL6, IL-1β and in total (IL6+IL-1β) do not differ significantly with the material. (Z, T or A)

At T2 only IL-1β differs significantly with the material, and if we analyze this in pairs we see that there is a significant difference between titanium and zirconium, with IL-1β being, on average, significantly higher in titanium (*p*-value = 0.023). The were no statistical differences in the other 2 pairs (zirconia-acrylic and titanium acrylic).

When using titanium as an abutment in IL6 here are no differences between T0 and T2.

In contrast, the IL-1β and the total IL (IL-1β +IL6) are significantly higher at T2 for the titanium abutment.

When we study the behavior on the acrylic, the conclusions are the same for the titanium and the zirconia on all indicators where IL-1β is significantly higher at T2,

although individually zirconia expresses significantly less IL-1β at T2 than Titanium.

The periodontal crevicular fluid (PCF) vs Perimplant crevicular fluid (PICF) component of the study mainly acted as one of the control groups (the other

was the blood samples) and highlighted what for me is one of the most relevant conclusions of this work.

When we analyze by time frame, we see that at T0 for IL6, the results are higher than PCF and in IL-1β the results are worse than that of a healthy tooth (PCF), which is understandable since implant placement (T0) induces an acute reaction while the periodontal sulcus has an established low intensity chronic infiltrate. (and, as a result, less expression of IL6 and higher expression of IL-1β than PICF)

At T0 for the titanium, zirconia and the acrylic, IL6 is, on average, significantly higher than the value of the PCF.

At T0 the values for IL-1β titanium, acrylic and zirconia are, on average, significantly, less than the value of the tooth.

But at T2, titanium expresses a significantly higher inflammation pattern than the periodontal sulcus of a healthy tooth, while zirconia shows a concentration similar to the periodontal crevicular fluid.

Breaking down the results for IL6 and IL-1β values for the titanium and the acrylic we found that at T2 IL6 is, on average, significantly higher than the value of the PCF, but in zirconia at T2, it is, on average similar to the value of the tooth

For IL-1β levels at T2, titanium is, on average, significantly higher than the value of the tooth, while acrylic and zirconia have statistically equal values to the IL-1β values of a healthy tooth.

We can conclude that the connective tissue experiences zirconia as a similar material to the tooth, making it more inert and not initiating a foreign-body reaction.

Another interesting control was to observe the impact of implant placement compared to the IL levels in blood at the time of surgery and most of these IL did not exist in the blood (or only in miniscule ammounts) at the time of implant placement. The fact is, it is surgical trauma that induces a rise in IL6 and IL-1β at T0.

Thus the overall inflammatory patterns are higher in titanium.

In marginal bone loss there is a tendency for titanium to express more loss than zirconia although we no correlation was found on a statistical level. What we did find was that, when a protocol of one abutment one time was usedon a platform switching implant, the MBL values were much lower than the success criteria described by Albrektsoonand using a zirconia abutment only improves that result.

Probably one of the strongest conclusions of this work, and one that probably has never been published in the literature is the impact of the residual gingival thickness on inflammatory levels and MBL.

In our study, we found that gingival height does not significantly influence marginal bone loss, either at MBL1 or MBL2. This is understandable, since MBL is multifactorial and to isolate just one variable would need an enormous sample size, which doesn´t mean that it has no impact, but rather that more studies are needed on this topic.

Howver, when it comes to inflammation at T0, in what is considered early healing, the height of tissue significantly influences the values of IL6, IL-1β and the total. On average, these indicators were significantly higher at a 3 mm tissue height than they were at 2 mm.

In all indicators at T2 height does not significantly influence IL-1β, IL6 and the total indicators, mainly because the periodontium is established, a situation which is very different from the acute healing at T0.

A number of interesting conclusions were drawn in the secondary outcomes.

In our work and, in relation to age and gender as final results we found that at T2 (8 weeks) age did not significantly influence IL-1β, IL6 and total values.

At T0, IL6 differs significantly with age, and, on average, IL6 is significantly higher at ≥65 years. The same conclusions apply for IL-1β and the total showing that at T0 patients older of equal to 65 years old tend to express more inflammation (IL6, IL-1β and IL-1β +IL6) in the early stages of implant placement than patients below 65 years old, despite that fact, after 8 weeks, Interleukin expression is the same in both groups.

For the secondary outcome with regard to inflammatory indicators at T2, IL-1β,

IL6 and in total, there is no significant difference between genders and at T0, IL6 is, on average, significantly higher in males.

The other inflammatory indicators do not differ significantly with gender.

Marginal bone loss is significantly influenced by gender: in our study we found, on average, that women experience greater bone loss than men

Gender significantly influences MBL2 meaning that more implants are exposed, and on average women lose more bone than men. However, if we look solely at the indicator, MBL1 is not significantly influenced by gender.

The position (maxilla vs mandible) in the final results on MBL inflammation and biomaterials indicates that only MBL2 differs significantly with the position, and in the maxilla, bone loss is, on average, significantly higher.

None of the 3 inflammatory indicators (IL6, IL-1β and total IL6 + IL-1β) at T2 differ significantly with position. The same conclusion can be drawn for T0 (baseline).

In our protocol no correlation was found between the duration of surgery and other variables and it was concluded that time does not significantly influence marginal bone loss and in no case, in our research, does the duration influence the indicated inflammatory variables.

The primary stability group showed that there are significant differences in stability between T0 and T2 and, on average, stability is significantly higher at T2 than at T0.

The results showed that at T0 and T2, stability is not significantly related to bone loss.

One of the first conclusions is that at T0 (baseline), implant stability is not significantly related to marginal bone loss (either MBL1 or MBL2), nor is it related to inflammation, namely at IL6, IL-1β and in total.

The overall conclusion of this thesis is that the autoimmune response is of great importance (and it is no myth) when alloplastic materials are placed in the oral cavity. When this happens, there is a mediated autoimmune response measured in IL-1β and IL6 when different biomaterials come into contact with oral connective tissue.

In a situation where there was supposedly “a bacteria free environment”, the titanium evokes a foreign bone reaction that triggers a rise in IL-1β inflammatory level.

Zirconia behavior was better and produced less inflammation, with behavior similar to a healthy tooth.

In an RCT where the confounding factors are adulterated it is our clinical recommendation (based on these results) that the use of titanium components in abutments should be reduced, in part due to the potential harmful effect on the health of the peri-implant tissues when compared to zirconia or cad-cam acrylic.

However, more trials should be undertaken to confirm these results.

Future?

In the future inflammatory parameters will for sure have a center role in oral implant pathology and many targets will be the IL.

Drug interaction will also target interleukins in order to refrain marginal bone remodeling and in some extreme cases refrain perimplantitis.

IL will change the paradigm of periodontology giving rise to new classifications that include inflammation as a target for periodontal/perimplant health.

CHAPTER 8. REFERENCES

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APPENDIX A. LICENSE FOR THE ANIMAL EXPERIMENT

REPÚBLICA PORTUGUESA

dgav

Ex.mo Senhor
Prof. Doutor João Manuel Mendes Caramês
Faculdade de Medicina Dentária da Universidade de Lisboa
Av. Columbano Bordalo Pinheiro nº 108, 7ºDto
1070 – 068 LISBOA

2016-04-21 010361

Nossa referência	Vossa referência	Vossa data
0421/000/000 /2016		

Assunto: **PROTEÇÃO DOS ANIMAIS UTILIZADOS PARA FINS EXPERIMENTAIS E/OU OUTROS FINS CIENTÍFICOS – PEDIDO DE AUTORIZAÇÃO PARA REALIZAÇÃO DE PROJECTO DE EXPERIMENTAÇÃO ANIMAL**

Na sequência do pedido efetuado por V. Exª no sentido de poder ser autorizada a realização do projeto experimental designado "**Resposta Perimplantar à colocação de pilares protéticos com diferentes materiais em implantes endósseos – estudo em modelo de ovelha**" de que é o investigador responsável, cabe-me informar que o mesmo foi avaliado de acordo com o Artigo 44º do Decreto-Lei nº 113/2013, de 7 de Agosto, relativo à "proteção dos animais utilizados para fins científicos".

Mais se informa V. Exª que esta Direção Geral, depois de esclarecidas as dúvidas que a sua análise nos levantou, o projeto em apreço recebeu uma **avaliação favorável** e foi **autorizado** de acordo com o nº 1, do Artigo 42º do mesmo diploma legislativo.

Para além disso, e dado os procedimentos a realizar aos animais serem classificados como "**Moderado**", é primordial fazer-se, no decorrer do desenvolvimento do projeto, uma adequada monitorização dos sinais de dor, sofrimento ou angústia dos animais submetidos aos procedimentos, por forma a poder fazer-se uma atualização sobre o nível de dor efetiva a que os mesmos possam ficar sujeitos, nomeadamente, de tipo "Severo", o que, a acontecer, implicará a sujeição do projeto a uma avaliação retrospetiva.

Finalmente, resta-me especificar, de acordo com o discriminado no nº 2, do Artigo 46º, do atrás referido Decreto-Lei, o seguinte:

- ***O utilizador que realiza o projeto:*** Diretor do Pólo de Investigação da Fonte Boa do INIAV;
- ***A pessoa responsável pela execução global do projeto e pela sua conformidade com a autorização do mesmo:*** Prof. Doutor João Manuel Mendes Caramês;
- ***O estabelecimento onde o projeto vai ser realizado:*** Pólo de Investigação da Fonte Boa, Santarém, do INIAV.

Com os melhores cumprimentos,

O Diretor Geral

Álvaro Pegado de Mendonça

DBEA/APM

APPENDIX B. ETHICAL COMITTE PERMISSION AND INFORMED CONSENT

U LISBOA

FACULDADE DE MEDICINA DENTÁRIA

Comissão de Ética para a Saúde (CES-FMDUL)

PARECER

A Comissão de Ética para a Saúde da Faculdade de Medicina Dentária da Universidade de Lisboa (CES-FMDUL), apreciou o pedido de parecer para a realização de um estudo intitulado ***"Resposta Perimplantar á colocação de pilares de cicatrização com diferentes materiais em implantes endósseos - ensaio clínico aleatorizado 1B"*** cujo investigador principal é no Senhor Professor Doutor João Caramês, e decidiu emitir **parecer favorável.**

Lisboa, 10 de fevereiro de 2016

O presidente da CES-FMDUL

(Professor Catedrático João Aquino)

Consentimento Informado

Este consentimento informado destina-se a todos os pacientes que preencham os requisitos e critérios de inclusão para o estudo *"Resposta Perimplantar á colocação de pilares de cicatrização com diferentes materiais em implantes endósseos - ensaio clínico aleatorizado-1B"*

Este estudo será realizado na Faculdade de Medicina Dentária da Universidade de Lisboa (FMDUL), no departamento de implantologia, dirigido pelo Prof.Doutor João Caramês e será coordenado pelo Investigador Dr. André Chen, Assistente convidado da FMDUL.

Foi seguido o modelo recomendado pela WHO-World Health Organization para consentimentos informados referentes a ensaios clínicos.

Parte I - Folha informativa

Parte II - Certificado de consenso (com assinatura, caso o doente aceite fazer parte do estudo)

Será dada uma cópia deste consentimento informado ao doente

Parte I - Parte Informativa

1-Introdução

Faculdade de Medicina Dentária da Universidade de Lisboa Departamento de Implantologia

O departamento de Implantologia da Faculdade de Medicina Dentária da Universidade de Lisboa tem como objectivos o ensino pós-graduado na área da Implantologia, através da prestação qualificada de serviços de Medicina Dentária à população, e o contributo para o desenvolvimento desta área do saber. Nos últimos anos novos materiais e técnicas têm sido introduzidos pela comunidade científica na prática clínica diária. Para avaliar a eficácia dos biomateriais utilizados correntemente como pilares de reabilitação (zirconia, titanio ou acrílico) propomos a sua participação neste estudo. Iremos fornecer informações detalhadas e convidá-lo/a a fazer parte deste estudo. Se tiver alguma questão, poderá esclarecê-la com um dos médicos participantes do estudo.

2- Objectivo da Investigação

Esta investigação pretende comparar três dos materiais mais utilizados em reabilitação sobre implantes (zirconia, titanio ou acrílico) e observar qual tem melhor desempenho em torno dos implantes. O nosso objectivo é saber, se o titanio é melhor, do que a zirconia, o acrílico fresado, ou o acrilico processado laboratorialmente, no que respeita á forma como a gengiva cicatriza em torno desse pilar de cicatrização.

3- Tipo de Intervenção

O estudo irá envolver quatro grupos. Após a colocação de cada implante, será colocado um pilar de cicatrização. Em cada grupo será escolhido aleatoriamente um dos quatro tipos de materiais. Um grupo receberá uma pilar de cicatrização em titanio, outro grupo em zirconia, outro em acrílico fresado (idealizado por computador e processado por uma máquina fresadora) e outro em acrílico laboratorial (processado pelo técnico de laboratório).

4- Seleção de participantes

Iremos convidar todos os pacientes que tenham implantes para colocar na zona posterior ao canino do maxilar inferior ou superior, com estrutura óssea suficiente, sem a necessidade de fazer procedimentos de regeneração.

5- Participação Voluntária

A sua participação neste estudo é totalmente voluntária. É sua, a escolha em participar ou não. Se optar por não participar neste projecto de investigação, pode á mesma realizar o procedimento á margem deste estudo.

6- Procedimento e protocolo

A literatura atual deconhece se um tipo de pilar é melhor do que outro no que diz respeito á cicatrização dos tecidos perimplantares (gengiva e osso), é preciso comparar os quatro. É importante que, nem o paciente nem o investigador tenham conhecimento sobre qual dos 4 pilares foi colocado. Esta informação estará nos nossos arquivos. Esta é a melhor maneira que temos para que o estudo não seja influenciado por aquilo que pensamos.

Após os implantes terem sido colocados juntamente com os pilares de cicatrização iremos proceder a medições espaçadas no tempo (abaixo ver tabela). Vão existir três tipos de medições : 1 - Radiográfica, iremos fazer radiografias para medir a quantidade de perda óssea ao redor de um implante ao longo das 8 semanas, 2- Estabilidade/ osteointegração, feita ás 8 semanas na consulta da impressão final, 3- Iremos também colher uma amostra do fluido gengival através da colocação de uma pequena tira de papel adsorvente no espaço entre a gengiva e o implante para poder medir a quantidade de mediadores inflamatórios.

Qualquer um destes procedimentos poderão ser considerados de rotina na implantologia actual e/ou sem consequências nocivas para o paciente.

7- Descrição do Protocolo e Programa de Consultas

Se concordar em participar neste estudo terá de obrigatoriamente comparecer aproximadamente 10 na FMDUL. Este número de visitas não é muito diferente do número de visitas regulares num tratamento convencional com implantes. As consultas assim como o que se vai fazer em cada uma delas está exemplificado no quadro abaixo

8- Riscos

Os riscos que existem ao entrar nesta investigação são os mesmos a que se expõe quando vai fazer um tratamento com implantes na zona do maxilar inferior/superior posterior, fora do âmbito deste estudo. Não iremos usar nada que não seja de uso corrente nos consultórios dentários em Portugal. O procedimento é igual ao procedimento que usaria em qualquer gabinete dentário ou bloco operatório onde se colocam implantes dentários.

O titanio é o material em são feitos os implantes e a maioria dos componentes de reabilitação. A zirconia é hoje usada para confeccionar coroas, estrutura para próteses ou pilares, e o acrílico é o material de eleição para a confecção de próteses provisórias quer sobre

implantes quer sobre dentes.

O risco da utilização destes materiais pode ser considerado não inerente ao estudo em si, mas sim inerente á própria medicina dentária, visto que o paciente pode ser alérgico a algum destes materiais, ainda que seja muito raro isso acontecer.

9- Confidencialidade

A informação que recolhemos a partir deste projeto de investigação será mantida em sigilo. As Informações recolhidas durante a pesquisa serão guardadas e ninguém, excepto os investigadores terão acesso ás mesmas.

10- Resultados

Os resultados desta investigação serão publicados em revista própria e tornados públicos para toda a comunidade cientifica, sendo que a sua participação será mantida em sigilo.

11- Direito a recusar ou a desistir

Não tem de participar nesta pesquisa, se não quiser fazê-lo não afetará o seu tratamento nesta instituição. Terá todos os benefícios que você teria na clínica. Pode interromper a sua participação no estudo em qualquer momento, sem perder qualquer dos seus direitos como paciente nesta instituição. O seu tratamento na clínica não será afetado de alguma forma.

12- Contacto

Caso tenha algumas perguntas pode perguntar agora ou mais tarde, mesmo depois do estudo ter sido iniciado. Se quiser fazer perguntas mais tarde, pode contactar por email - tsouchen@gmail.com, o investigador principal.

Parte II - Certificado de consentimento

Declaração do consentimento do participante:

Eu li as informações acima, ou ele foi-me lido. Eu tive a oportunidade de fazer perguntas sobre o assunto e todas as perguntas que fiz foram respondidas para minha satisfação. Concordo voluntariamente em participar como participante neste estudo

Nome do Participante_____ Assinatura do participante _____
Data_____

Dia/Mês/ano

APPENDIX C. RCT REGISTER SHEET

ClinicalTrials.gov PRS
Protocol Registration and Results System

ClinicalTrials.gov Protocol Registration and Results System (PRS) Receipt
Release Date: April 29, 2017

ClinicalTrials.gov ID: NCT01961635

Study Identification

Unique Protocol ID:	II-01
Brief Title:	Healing of Bone/Soft Tissue to Different Abutment Biomaterials and the Impact on Marginal Bone Loss
Official Title:	Healing Response if Peri-implant Tissues to Different Abutment Materials - Double-blinded Randomized Clinical Trial
Secondary IDs:	

Study Status

Record Verification:	April 2017
Overall Status:	Completed
Study Start:	January 2016 []
Primary Completion:	March 23, 2017 [Actual]
Study Completion:	March 23, 2017 [Actual]

Sponsor/Collaborators

Sponsor:	Instituto de Implantologia
Responsible Party:	Principal Investigator Investigator: Andre Chen [achen] Official Title: Msc Affiliation: Instituto de Implantologia
Collaborators:	

Oversight

U.S. FDA-regulated Drug:	
U.S. FDA-regulated Device:	
U.S. FDA IND/IDE:	No
Human Subjects Review:	Board Status: Approved Approval Number: 10/02/2016 Board Name: A Comissão de Ética para a Saúde da Faculdade de Medicina Dentária da Universidade de Lisboa (CES-FMDUL) Board Affiliation: A Comissão de Ética para a Saúde da Faculdade de Medicina Dentária da Universidade de Lisboa (CES-FMDUL) Phone: +351217 922 626 Email:

	Address: Cidade Universitária 1649-003 Lisboa | Portugal
Data Monitoring:	No
FDA Regulated Intervention:	No

Study Description

Brief Summary:	In patients that require a dental implant, does zirconia compared to titanium, or cad-cam acrylic abutments, provide less inflammation, marginal bone loss or infection during the osseointegration period ?
Detailed Description:	Place dental Implants and zirconia, titanium, acrylic or cad-cam acrylic abutments, torque to 20 n/cm2 Evaluate Changes in inflammatory levels from T0 (baseline) to T7-12 weeks (measure intermediate points at T0 (baseline),,T1-8 Weeks and T2-12 weeks) Also evaluate outcomes marginal bone loss, gingival height levels and osseointegration

Conditions

Conditions:	Bone Loss Inflammation
Keywords:	Dental Implants Zirconia Abutments Titanium abutments Acrylic abutments Gingival Healing Inflammation Bone loss

Study Design

Study Type:	Interventional
Primary Purpose:	Treatment
Study Phase:	N/A
Interventional Study Model:	Parallel Assignment
Number of Arms:	3
Masking:	Triple (Participant, Care Provider, Outcomes Assessor)
Allocation:	Randomized
Enrollment:	60 [Actual]

Arms and Interventions

Arms	Assigned Interventions
Experimental: Zirconia Abutments Place zirconia one-time one-abutment in subcrestal platform-switch dental implants on the day of implant installation, torque 20 n/cm2	Device: dental implants place implant according fabricant guidelines, 2mm sub-crestally Other Names: • T3 - Biomet 3I Implants

Arms	Assigned Interventions
	• Platform-Switch 4.1 • Abutment Diameter 3.25 Device: Zirconia abutments place zirconia abutment on the day of implant placement Other Names: • Zirconia cad-cam abutment • 3.25 Abutment connection Procedure/Surgery: Subcrestal place implant 2mm below crestal bone Device: platform-switch abutment platform is narrow than implant diameter Other Names: • platform-switch Procedure/Surgery: one-time one-abutment place the final abutment on the day of surgery and not remove it Other Names: • one time abutment • final abutment on the day of surgery Procedure/Surgery: Torque 20 n/cm2 the amount of torque applied to the abutment Other Names: • manual torque
Experimental: Titanium Abutments Place titanium one-time one-abutment in subcrestal platform-switch dental implants on the day of implant installation, torque 20 n/cm2	Device: dental implants place implant according fabricant guidelines, 2mm sub-crestally Other Names: • T3 - Biomet 3I Implants • Platform-Switch 4.1 • Abutment Diameter 3.25 Device: Titanium Abutments place titanium abutment on the day of implant placement Other Names: • Titanium Encode - biomet 3I Implants • 3,25 abutment connection Procedure/Surgery: Subcrestal place implant 2mm below crestal bone Device: platform-switch abutment platform is narrow than implant diameter Other Names: • platform-switch Procedure/Surgery: one-time one-abutment place the final abutment on the day of surgery and not remove it Other Names: • one time abutment

Arms	Assigned Interventions
	• final abutment on the day of surgery Procedure/Surgery: Torque 20 n/cm2 the amount of torque applied to the abutment Other Names: • manual torque
Experimental: Cad-Cam Acrylic abutments Place cad-cam acrylic one-time one-abutment in subcrestal platform-switch dental implants on the day of implant installation, torque 20 n/cm2	Device: dental implants place implant according fabricant guidelines, 2mm sub-crestally Other Names: • T3 - Biomet 3I Implants • Platform-Switch 4.1 • Abutment Diameter 3.25 Device: cad-cam acrylic abutments place cad-cam acrylic abutment on the day of implant placement Other Names: • Cad-cam acrylic abutment • 3.25 platform Procedure/Surgery: Subcrestal place implant 2mm below crestal bone Device: platform-switch abutment platform is narrow than implant diameter Other Names: • platform-switch Procedure/Surgery: one-time one-abutment place the final abutment on the day of surgery and not remove it Other Names: • one time abutment • final abutment on the day of surgery Procedure/Surgery: Torque 20 n/cm2 the amount of torque applied to the abutment Other Names: • manual torque

Outcome Measures

Primary Outcome Measure:

1. Gingival Inflammatory Changes from baseline to 12 weeks
   measure the amount of cytokines and interleukines (interleukin-8 (IL-8), macrophage inflammatory protein-1 (MIP-1 beta), interleukin-1 (IL-1 beta), interleukin-6 (IL-6), matrix metalloproteinase-8 (MMP-8) and matrix metalloproteinase-9 (MMP-9), metallopeptidase inhibitor 1 (TIMP-1), vascular endothelial growth factor (VEGF)), around different implant abutment materials, zirconia,titanium, acrylic, and cad-cam acrylic

   [Time Frame: Measure - T0 Implant installation T1- 1week post-surgery T2-two weeks T3-3weeks T4-4weeks T5-6weeks T6-8weeks T7-12 weeks]

Secondary Outcome Measure:

2. Marginal Bone Loss

Parallel periapical standardized radiographs to measure bone position in relation to implant platform, in zirconia,titanium, acrylic, and cad-cam acrylic

[Time Frame: measured at 12 weeks post-implant installation]

3. Gingival height
measure with periodontal probe distance from implant platform to the most coronal point of gingival margin mesial, distal, buccal and lingual,in zirconia,titanium, acrylic, and cad-cam acrylic

[Time Frame: 12 weeks post-implant installation]

4. Osseointegration
compare survival rate of dental implants rehabilitated with zirconia,titanium, acrylic, and cad-cam acrylic abutments

[Time Frame: 12 weeks post-implant installation]

5. Height from gingiva to abutment
height from gingival margin to most coronal point of the abutment in zirconia, titanium, acrylic and acrylic cad-cam abutments

[Time Frame: 12 weeks post implant installation]

Eligibility

Minimum Age:	18 Years
Maximum Age:	
Sex:	All
Gender Based:	
Accepts Healthy Volunteers:	Yes
Criteria:	Inclusion Criteria:

- Single unit implant rehabilitation
- Maxilla and mandible
- Must accept treatment plan
- Must sign informed consent
- dental extraction performed at least 3 month prior
- Must have at least 6 mm of residual bone
- Absence of oral lesions
- keratinized tissue must be present

Exclusion Criteria:

- If smoking and/or other drug addiction is present
- If local anesthetic allergy is present
- Patient subjected to chemical or radiotherapy
- if Hepatic disease is present
- If immunodepression is present
- If Pregnancy is present
- If Diabetes is present
- If Heart disease is present

Contacts/Locations

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Study Officials: João Caramês, Phd
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Helena Francisco, Phd
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Locations: Portugal
Faculdade de Medicina Dentária de Lisboa
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Principal Investigator: Andre Chen, Msc
Principal Investigator: João Caramês, Phd
Sub-Investigator: Elena Cervino, Msc
Sub-Investigator: Helena Francisco, Phd
Sub-Investigator: Canta João, Msc

IPDSharing

Plan to Share IPD: Undecided

References

Citations:

Links:

Available IPD/Information:

APPENDIX D. LIST OF FIGURES

APPENDIX E. LIST OF TABLES